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STUDIES OF THE PHYSIOLOGICAL IMPORTANCE OF THE MINERAL ELEMENTS IN PLANTS

THE VARIATION IN POTASSIUM CONTENT OF MAIZE LEAVES DURING THE DAY

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(With 5 figures in the text)

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INTRODUCTION

An earlier paper of this series (Penston, 1935) describes the daily changes in potassium content of potato leaves. It was shown that significant variations occur in the weight of potassium in any given leaf at different times of the day, and that they are related to changes in dry matter and water content. Potassium reaches its maximum at about 3.0 p.m. when dry-weight accumulation due to photosynthesis is also at its highest. Towards evening when the elaborated food materials are being translocated from the leaf to other parts of the plant, potassium also moves out of the leaf. Four quantities were determined: water, dry matter, potassium and

residual ash; and all, plotted graphically, show a more or less rhythmic rise and fall during the hours of daylight.

The present paper is concerned primarily with a similar investigation of the daily changes occurring in maize leaves. This plant differs considerably, in many respects, from the potato, and so should provide a good check as to whether the earlier results are general or not; that is to say, whether the state of affairs found in the potato leaves can be considered as holding for all metabolizing leaves of plants grown under natural conditions.

In addition to determining the daily changes in the quantities mentioned, some attempt has been made to correlate them with the stage of development of the leaves concerned and of the rest of the plant. For this purpose records have been taken, at regular intervals throughout the season, of the dry weights of all the aerial portion of the plant.

MATERIALS AND METHODS

The maize plants were grown on a plot of ground at Sydenham in 1935. The soil was good, and there was no danger of mineral deficiency. The weather during the summer was on the whole very favourable to the growth of these plants, as the maximum dry weight will show; and throughout the season their development appeared to be quite uniform.

The plant samples. Seven samples of the aerial parts of plants were collected between 18 June and the end of September. The number of plants gathered ranged from thirty-five on the first occasion to ten on the last, when, owing to the fact that the plants were 7 ft. high, very bulky, and the laboratory some miles away, no more could conveniently be handled. It was thought that as the growth of the plants had been so uniform, this would not be too small a number to take. The selection of plants was made at random, and the samples were collected at 2.30 p.m. on each occasion, to avoid errors due to fluctuation of the dry weight as a result of photosynthesis. The fresh weight of the material was determined immediately, after which it was separated into leaf blades, leaf sheaths, stems, and flowers and fruits when these were present. In the laboratory the separate portions were dried in an electrical oven at 100° C. for 48 hr., weighed, ground to powder and stored.

The leaf samples. Three collections of leaves were made, one on 15 July and two on 9 August. Each of these experiments represents a series of samples of forty-five to fifty blades. All the leaves in any

one series of samples were of the same age, but leaves of different ages were taken in the three experiments. The samples, selected at random, were collected at hourly intervals from 10 a.m. to 5.0 p.m. summer time. The experiments could not be continued longer because of the difficulty of getting the samples back to the laboratory and into the drying oven before nightfall. The fresh weight of the leaves was determined immediately after collection, then they were wrapped in cellophane and stored in a cool place until all were ready to be taken away. Dry-weight determinations were made in the usual way, the dried matter being then ground to powder and stored in air-tight containers for subsequent determinations of ash and potassium.

The analyses. In each case triplicate portions of 0·5 g. of dry material were ashed in porcelain crucibles at a dull red heat, weighed, then treated with HCl, dried again, dissolved in boiling distilled water and made up to 50 c.c. Potassium was determined by the cobalt hexanitrite method. Only one modification was introduced to the method previously described (James & Penston, 1933). The precipitate, instead of being centrifuged, was filtered off on to a small Jena scintered glass filter funnel, where it could be thoroughly washed with 70% alcohol to remove the reagent before being redissolved in distilled water for the volumetric estimation with permanganate.

Experimental results

Plant samples. The stage of development at times of sampling can be summarized as follows: The seed was sown on 11 May and by 1 June (3 weeks later) the plants were just visible above ground. Four leaves were counted on the main stem on 18 June (5 weeks), nine leaves on 2 July (7 weeks), twelve leaves on 16 July (9 weeks), the total number of leaves on the main stem, eighteen, in the variety used, had all been formed, and the terminal tassels and some of the axillary female branches were beginning to develop on 2 August (11–12 weeks). Thus leaf formation on the main axis extended over a period of 11 weeks. They were not all fully developed, however, until the end of August (15 weeks). By the end of September the cobs were ripe and many of the leaves showed signs of senescence (20 weeks).

In Table I are given the results of the dry-weight determinations of the separated aerial portions of the seven samples collected between 18 June and 31 September. They are expressed in grams per 100 plant parts. The total dry weights represent the summations of the weights of individual organs. The total fresh weight also given was, as

previously stated, determined directly on the samples when first gathered. The flowering portions were weighed for the first time on 2 August, on this and subsequent dates the second figure in brackets under the total dry-weight column represents total dry weight less weight of reproductive portion. In this way it can be seen that loss in weight occurred in the vegetative regions during the last month when the cobs were ripening. By the end of September the fully ripened cobs had a weight equivalent to just over a quarter of the total weight of the aerial parts of the plant.

TABLE I. *Maize plant samples: dry weights of the aerial portions expressed in grams per* 100 *plant parts*

Date of sampling	Dry weights in grams: Leaf blades	Leaf sheaths	Stems	Flowers etc.	Total	Fresh weight total
18 June	2·6	2·25*		—	4·8	53·0
2 July	52·6	21·67*		—	74·3	688·0
16 July	658·1	238·3	195·8	—	1092·2	11800·0
2 Aug.	1751·1	1665·3	1217·5	75·5	4709·4 (4633·9)	55850·0
13 Aug.	4860·5	1936·0	2244·0	907·5	9947·0 (9039·5)	76261·5
27 Aug.	8733·3	4408·3	8083·3	3288·3	24513·4 (21225·1)	159016·0
30 Sept.	6460·0	5930·0	7840·0	7470·0	27700·0 (20230·0)	188260·0

* Stems and sheaths were weighed together on these dates.

The dry-weight values are represented graphically as log curves plotted against time in Fig. 1. The curves consist of three distinct parts: a steeply ascending region indicating the rapid growth of the main axis and its leaves; a middle, less steeply ascending period when the majority of these leaves were mature and increasing only very slightly in weight, while the laterals and flowers were probably responsible for most of the growth; and a final period when the curves begin to fall off, here the leaves were becoming senescent and only the fruits were increasing in weight.

Leaf samples. Table II summarizes the results of the determinations of water content, dry matter, potassium and residual ash, in the three series of daily samples of leaves. These results have been expressed as grams weight per hundred leaves (calculated from the weights of the 45–50 leaves collected in each sample at the times given), as this is the best method of showing and comparing the changes which occur in a leaf or other organ.

The results are given in graphical form in Figs. 2, 3 and 4. The first series on 15 July (Fig. 2) represents the fifth leaf up from the base of the main stem; the actual age of this leaf was between 3 and 4 weeks. The second and third series on 9 August (Figs. 3 and 4)

Fig. 1. (A) Dry weights of the samples of aerial portions of maize plants, expressed as log *W* against age in weeks. (B) Log *W* against age in weeks of the three leaf samples collected on 15 July and 9 August.

represent the tenth and the eighth leaf up from the base of the main stem respectively, the former was 5–6 weeks, the latter 7 weeks old. These three leaves were, therefore, at different stages of development, the first still actively growing, the third more or less mature, and the second somewhere in between the two. A comparison of the dry weights at 11 o'clock in the morning, the minimum weight in each

TABLE II. *Water, dry matter, residual ash and potassium, in maize leaves. Weights in grams per* 100 *leaves*

Time (G.M.T.)	H_2O	D.M.	Ash-K	K
Exp. I, 15 *July* 1935. Fifth leaf up from the base of the main stem. Age 4 weeks:				
9 a.m.	329·0	52·5	5·85	2·20
10 ,,	316·9	51·6	5·66	1·972
11 ,,	224·52	37·0	4·47	1·33
12 noon	245·42	41·6	4·507	1·523
1 p.m.	277·78	48·2	5·06	2·09
2 ,,	289·17	50·83	5·215	2·305
3 ,,	246·45	45·25	4·795	1·965
4 ,,	276·67	48·33	5·05	2·05
Exp. II, 9 *August* (*i*). Tenth leaf up from the base of the main stem. Age 5–6 weeks:				
9.15 a.m.	996·0	223·4	27·98	8·32
10.15 ,,	1088·4	253·3	26·38	12·2
11.15 ,,	860·6	203·3	25·67	8·03
12.15 p.m.	925·9	218·3	23·55	9·75
1.15 ,,	998·7	236·3	26·82	11·58
2.15 ,,	969·4	225·0	24·72	9·79
3.15 ,,	1079·0	255·0	26·45	12·45
4.15 ,,	1033·9	259·9	26·45	12·15
Exp. III, 9 *August* (*ii*). Eighth leaf up from the base of the main stem. Age 7 weeks:				
9 a.m.	1232·4	321·6	33·5	13·3
10 ,,	1421·3	359·7	38·95	17·15
11 ,,	1219·2	302·8	26·0	11·9
12 noon	1278·6	330·4	31·98	14·82
1 p.m.	1298·2	332·8	33·35	14·5
2 ,,	1257·2	322·8	29·55	11·65
3 ,,	1396·5	351·5	38·3	15·6
4 ,,	1411·0	330·0	30·35	12·95

case, will emphasize this point (i) 37 g., (ii) 200 g., (iii) 300 g. The log curve of these weights, drawn in the corner of Fig. 1, also shows how the rate of increase in dry weight is falling off with age.

The reason for collecting two sets of leaves on 9 August, was to get a comparison in the changes in dry matter, etc., in leaves of different ages under similar external conditions controlling transpiration and photosynthetic activity. Allowing for the slight difference in time at which the samples were collected, it is interesting to find that the general trend of the variations is substantially the same in both sets of leaves.

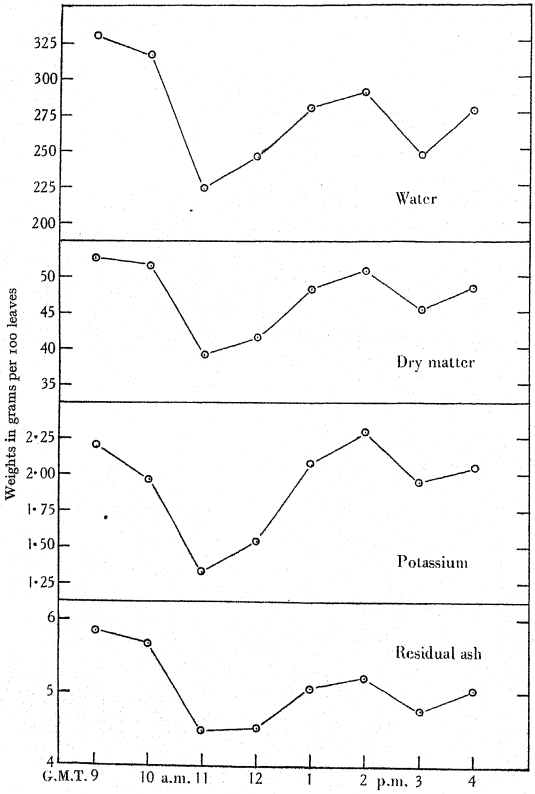

Fig. 2. Variations in water, dry matter, potassium and residual ash content of maize leaves, expressed in grams weight per hundred leaves: (i) the fifth leaf up from the base of the main stem.

DISCUSSION

Changes in water, dry matter, residual ash and potassium content of the maize leaves

Definite fluctuations in water content occur during the period of observation in all three experiments. It is seen in Figs. 2, 3 and 4,

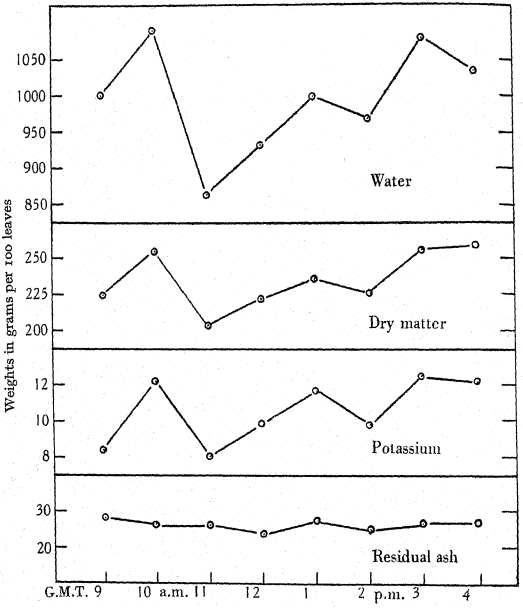

Fig. 3. Variations in water, dry matter, potassium and residual ash content of maize leaves, expressed in grams weight per hundred leaves: (ii) the tenth leaf up from the base of the main stem.

that the leaves contain least water at eleven in the morning. This is evidently the result of the interaction of factors such as high temperature and light intensity increasing transpiration, and soil factors limiting absorption. Water content of the leaves rises again between

11 and 2 p.m. in the first experiment and between 11 and 1 p.m. in the second and third, to be succeeded by a further small loss and subsequent gain later in the afternoon. Although water content thus increases after 11 o'clock, it does not, in the period examined, equal the amount present prior to that hour. The dry weight and potassium

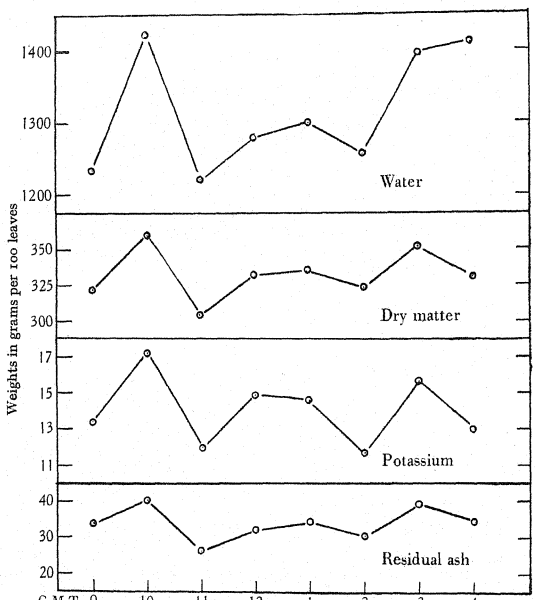

Fig. 4. Variations in water, dry matter, potassium and residual ash content of maize leaves, expressed in grams weight per hundred leaves: (iii) the eighth leaf up from the base of the main stem.

curves reveal similar changes to the curves for water content: a morning increase in weight followed by a loss at 11 o'clock, after which they rise again in the afternoon. Movement of potassium corresponds a

little more closely with water than does dry matter. The residual ash in the first and third leaves also shows considerable changes similar to the other substances, but in the second leaf these are not so well marked.

Relation of the changes to the stage of development of the leaves

An analysis of the fluctuations reveals one or two interesting points. For instance, the changes in weight between the morning maximum and 11 a.m., and again between 11 a.m. and the afternoon maximum can be compared (*a*) as change in weight in grams and (*b*) as percentage change in weight based on the minimum value. In Table III this has been done for water, dry matter and potassium.

TABLE III. *Magnitude of the changes in weight in* H_2O, *dry matter and* K *from the morning maximum to the minimum at* 11 *a.m.* (*a.m.*) *and from* 11 *a.m. to the afternoon maximum* (*p.m.*) *compared in the three leaves* (*a*) *as change in weight in grams,* (*b*) *as percentage of the minimum weight*

			Water		Dry matter		Potassium	
No. of exp.	Age of leaf in weeks	Period	(*a*) Wt. in g.	(*b*) %	(*a*) Wt. in g.	(*b*) %	(*a*) Wt. in g.	(*b*) %
I	3–4	a.m.	104·48	46·5	15·5	41·9	0·87	65·5
		p.m.	64·65	29·8	13·8	37·3	0·98	73·4
II	5–6	a.m.	227·8	26·5	50·0	24·6	4·17	52·0
		p.m.	218·4	25·4	56·6	27·9	4·42	55·0
III	7	a.m.	202·1	16·6	56·9	18·8	5·25	44·0
		p.m.	192·8	15·8	48·7	16·1	3·7	31·1

Bearing in mind that the first leaf was sampled under different external conditions from the other two, the comparison shows that the changes in weight of the three substances increase as the leaf nears maturity. In the case of dry matter for instance, the greatest change in the first leaf is about 15 g., and in the second leaf about 56 g. Clearly then, in a growing leaf the magnitude of the changes will bear some relation to the age and size of the leaf. When however, the nearly mature second leaf is compared with the mature third leaf, the magnitude of the changes is seen to be of the same order; which means that beyond a certain stage of development they will tend not to increase much further with age.

Using the second method of comparison, namely expressing the maximum fluctuation as a percentage of the minimum weights it is

found that the values for all three substances fall off with age of the leaf. The percentage increases are very large in the youngest leaf, for example dry weight about 40%, potassium about 70%, above the minimum weight, whereas in the mature leaf the corresponding values have fallen to 18 and 40%. By this method of comparison therefore, it would appear that relative to size, the photosynthetic activity and capacity for absorption of water and salts is greater the younger the leaf, because it does not take into account the increase in dry weight due to the building up of permanent tissues, or of water and mineral elements which are retained by the increased number of living cells as the leaf grows older. Thus too much emphasis cannot be given to the fact that in all comparisons based on percentage values the age of the organ should always be taken into account.

The nature of the relationship between potassium and stage of development of the leaf can be brought out by another method of analysing the results. If the minimum daily potassium weight in any of the three leaves is taken as representing as nearly as possible the basic weight required by the tissues of that leaf at its particular stage of development, then the difference in the basic weights between the 3–4 weeks and the 5–6 weeks old leaves represents the amount of potassium which has been absorbed during the two weeks' growth. The basic weights of these two leaves are 1·33 and 8·03 g. per 100 leaves, respectively (see Table II), the difference is therefore 6·7 g. and works out at an average daily increase during the two weeks of 0·478 g. per 100 leaves. Examining the daily fluctuations in potassium in the light of this theoretical daily rate of increase, it will be seen that in the 3–4 weeks old leaf between, for example, 12 and 1 o'clock, or between 2 and 3 o'clock (see Table II), the change in 1 hr. is equal to the whole of the average increment for the day, whilst in the 5–6 weeks old leaf the increase from a quarter past twelve to a quarter past one is four times the average daily increment. Even in the mature 7 weeks old leaf which has reached a stage of development when daily increase as a result of growth has become very small, changes equivalent to an increase of 40% above the minimum content can occur in the space of a few hours.

These points indicate that mineral elements such as potassium, can be absorbed into the leaf during certain hours of the day, in quantities exceeding the requirements for growth of the leaf itself; and secondly, that the power to absorb under conditions of metabolic activity, e.g. photosynthesis, continues past the growing period when increase in structural material is taking place.

The nature of the relationship between potassium and water, and potassium and dry matter

Potassium in the plant is almost entirely water soluble, and although the changes in both these substances during the day are considerable, the percentage value K/H_2O is relatively constant. The values lie between 0·59–0·75% in the first leaf, 0·8–1·2% in the second, and 0·8–1·21% in the third (Fig. 5). When potassium is expressed as a percentage of the dry weight, the results indicate that there is a slight disposition for potassium to accumulate in excess of dry matter while the latter is being built up in the leaf, and to leave the leaf in greater relative quantity than dry materials when translocation is being carried on (Fig. 5, between 11 a.m. and 1 p.m. and between 10–11 a.m.). The values of the percentage K/D.W. lie between 3·5 and 4·9% whatever the age of the leaf.

The close relationship between water and potassium suggests one of two things: either that water content is controlling the movement of potassium into and out of the leaf in such a way that the concentration in the cells is kept within certain limits, in which case osmotic pressure may be an important subsidiary controlling force; or that other forces which operate towards absorption and movement of ions, are themselves primarily controlled by water content. In the latter case, for instance, the decreasing water balance between 10 and 11 o'clock, by affecting stomatal opening and carbon dioxide intake, may tend to check photosynthesis. Thoday (1910) found that turgid leaves of *Helianthus annuus* carried on photosynthesis approximately ten times more rapidly than did leaves which were wilted to drooping. With this falling off in photosynthesis, secondary changes would probably take place, in, for example, H-ion concentration, which would lead to hydrolysis of starch if it had been formed, and subsequent translocation of materials from the leaf, leading to the observed loss in dry weight. These changes in metabolic activity may then be the direct cause of the migration of potassium. In other words, while the cells of the leaf are well supplied with water they are able to carry on photosynthesis and to accumulate ions such as potassium, but with fall in water content, active anabolism tends to cease and leads to changes in the state of the cells such that they can no longer retain cations already absorbed.

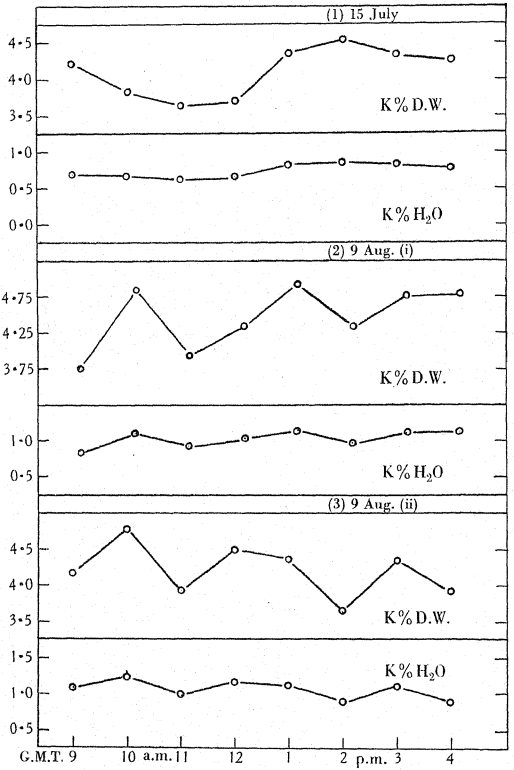

Fig. 5. Potassium content expressed as a percentage of dry weight and H_2O content in the three series of maize leaf samples.

SUMMARY AND CONCLUSIONS

1. Daytime changes in dry matter, water, residual ash and potassium content, in 3–4, 5–6 and 7 weeks old leaves of maize, are recorded. The results are expressed as grams weight per hundred leaves.

2. In all the leaves changes in these substances occur. They increase in the early morning, fall to a minimum value about 11 a.m., and rise again in the afternoon.

3. The general nature of the daily changes in maize leaves is found to be similar to what has already been described for leaves of the potato plant.

4. The magnitude of the changes, expressed as weight in grams, increases with age while the leaf is still actively growing, up to a time near maturity after which it no longer increases in proportion to the basic weight of the leaf. When, however, the changes are expressed as a percentage of the minimum weight of the leaf, there is a continuous falling off with age.

5. The changes in potassium and other mineral elements are considerable, whatever the age of the leaf, and are closely related to the changes in dry matter and water content. It is concluded that absorption and movement of mineral elements in leaves during the day is dependent upon the rate of metabolic activity.

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EXPERIMENTAL TAXONOMY

II. INITIAL POPULATION DIFFERENTIATION IN *PLANTAGO MARITIMA* L. OF BRITAIN

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(With 4 figures in the text)

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I. Introduction

It is customary to regard the species unit as the convenient starting point for evolutionary discussion. Doubtless this practice has been favoured because the species, notwithstanding its numerous interpretations, does on the whole approximate more nearly to an evolutionary entity than do the less well-defined units of lower rank. The *species* and the *variety*, however, differ only in degree, and there is actually no valid reason why the latter should not also be employed for the elucidation of evolutionary problems. On the basis of the accepted criteria it may sometimes be difficult to decide whether certain observable differences really warrant taxonomic recognition, and it is often a matter of personal preference whether species or varietal rank is awarded to a group. But there is no definite reason why demonstrable natural grouping should inevitably be relegated to the units of traditional taxonomy a new terminology resulting from a new orientation of the biological facts might be more appropriate. For instance it might be demonstrated by field and garden

experiment that the initiation of population differentiation is more important biologically than the magnitude of the resulting morphological differences: in this case the unit could be based on the causal factors, factors perhaps external to the plants themselves. The degree of morphological distinctness would under these circumstances have only a diagnostic value and no taxonomic status. Dependable criteria for the classification of initial population differentiation are undoubtedly difficult to obtain in the herbarium, and in consequence Hall (1929) proposed that small units should be described only in a numbered or lettered list of minor variations, and that they should be left in this provisional category until their status could be determined by experiment or other exact methods. Hall's suggestion has much to recommend it because in this way the known facts would be made available to the experimentalist without the systematist having to "commit himself when evidence is lacking" and "without introducing false concepts as to relationships".

In the first paper of this series (Gregor *et al.* 1936) it was advocated that a separate taxonomic system should be developed for recording the results of the experimental analysis of wild populations, because it was felt that any immediate attempt to absorb the experimental results into the existing system would be undesirable and could only lead to confusion. The present paper gives the results of an attempt to determine the biological significance of the initial character groupings which differentiate wild populations, and to illustrate a possible way of expressing taxonomically the findings of experimentation.

To establish the arrangement of characters in Nature, however, it is first necessary to obtain *representative* samples from the wild, and second to minimize the effect of fluctuating variability. Reliable samples of local populations are generally more easily obtainable than samples representative of a whole species. For this reason subspecific assemblages are to be preferred for the study of initial differentiation within an intrafertile group. The sea plantains of Britain (*Plantago maritima* L.) afford excellent material not only because the members of the population are interfertile, but because while the distribution round the coastline of the mainland is continuous, that of the adjacent islands and of the inland mountains is discontinuous. In an introductory paper (Gregor, 1930) it was shown that population differentiation could occur in the absence of spatial isolation, but at that time little information was available regarding the number and variability of characters affected by ecological separation. Information was then also lacking concerning the relative

effects of ecological and geographical isolation on the magnitude of population differences. Thirty-two population samples collected from habitats in Britain have now been grown in the experimental garden at Edinburgh and 100 or more plants of each have been studied by the methods previously described (Gregor *et al.* 1936). In addition a number of smaller samples were examined, but the data relating to these have not been included in the accompanying tables.

The distribution of Plantago maritima *in Britain*

The distribution of *Pl. maritima* in Britain is general throughout the coastal regions, but inland it occurs only in localized stations. In this it is remarkably similar to *Armeria vulgaris* Willd. which is one of the few other species in which these conditions obtain. For a better understanding of how this distribution has come about, it is of interest to note the present occurrence of these two species in Iceland. There, while they again occur generally on the coast, they are also common inland, although they are absent from the nunataks explored by Steindór Steindórsson. It is therefore likely that in Britain *Plantago maritima* spread post-glacially from the coastal districts to the higher mountains of Scotland and England subsequently being displaced from the intermediate regions. The occasional establishment in suitable habitats of migrants from the coastal regions may still take place, but it seems more probable that the mountains are the loci from which most present-day inland migrations radiate.

In the British mountain habitats *Pl. maritima* grows on thinly populated rock ledges, and at lower elevations it is most commonly found among boulders and escarpments in the vicinity of streams, some of these habitats being of very recent origin. No records are available as to the permanency of the lowland populations, but in all probability they are displaced by more aggressive species as the habitats become stabilized. Shade, rather than altitude, seems to be the limiting factor to lowland establishment, as is evident from the fact that at low elevations in regions of tall vegetation *Pl. maritima* is found only on the margins of streams and paths. Actually seed production is more abundant in the lowland habitats than in the subalpine (3000 ft.) zone where the plants are more or less scattered.

Since streams leading from mountain habitats provide the most probable lines of dispersal, plantain populations found on the banks of streams having a common mountain source have been

regarded, for the purposes of this investigation, as having a similar locus of dispersion; only habitats which are topographically unconnected have been considered as truly isolated. The inland population of the sea plantains of Britain has been regarded by White (1869) as representing three species: *Pl. maritima* L., *Pl. alpina* L. and *Pl. serpentina* Vill., but there seems to be no justification for making such distinctions, and the whole population should be considered as belonging to a single species, *Pl. maritima* L.

In contrast to the restricted inland distribution the coastal distribution is continuous. *Pl. maritima* has been recorded from every one of Watson's (1883) vice counties which possesses a coastline. In this paper coastal populations from the Western Isles are not treated with the coastal populations of the mainland on account of their geographically isolated position. In Fig. 1 the inland habitats from which samples were taken are indicated by black circles, hatched circles mark the position of the coastal populations sampled, while black triangles denote other inland records. The following samples have been critically studied in the experimental garden:

A. *Inland.*

I. Ben Lui locus (Perthshire):

PMN 17, collected 23. x. 27 at 500 ft., from the eroded shore of Loch Dochart. (Examined 1931.)

PMN 18, coll. 5. ix. 28 at 800 ft., from gravelly alluvium near Coninish. (1930.)

PMN 28, coll. 5. ix. 28 at 2000 ft., from banks of a stream. (1930.)

II. Ben Cruachan locus (Argyllshire):

PMN 16, coll. 23. x. 27 at 100 ft., from the banks of the river Awe. (1929.)

PMN 38, coll. 12. ix. 29 at 700 ft., from a *Festuca* pasture. (1933.)

PMN 39, coll. 11. ix. 29 at 120 ft., from rocky shore of Loch Awe. (1933.)

III. Unconnected habitats:

PMN 21, coll. 29. ix. 28 near Blair Atholl (Perthshire) at 450 ft., from gravelly alluvium adjacent to the river Garry. (1931.)

PMN 40, coll. 9. ix. 29 in Glen Shiel (Ross-shire) at 70 ft., from the banks of the River Shiel. (1934.)

PMN 55, coll. 10. ix. 30 on Carrick Hill (Ayrshire) at 750 ft., from areas adjacent to road. (1933.)

PMN 90, coll. 12. ix. 33 near Gillerthwaite, Ennerdale (Cumberland), at 500 ft., from a rocky hillside. (1936.)

B. *Island.*

I. Lewis (Hebrides):

PMN 47, coll. 6. ix. 30 near Stornoway from coastal waterlogged mud. (1932.)

PMN 48, coll. 7. ix. 30 near Stornoway from partially populated sea cliff. (1932.)

PMN 49, coll. 8. ix. 30 near Valtos from a meadow above highest tide mark. (1932.)

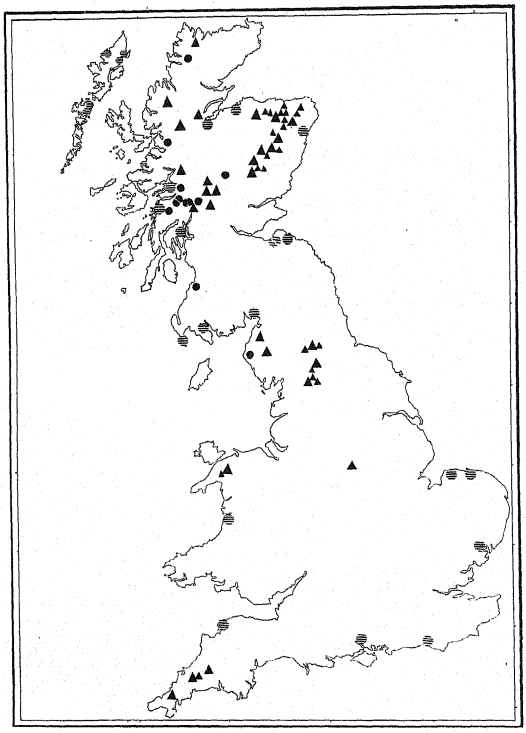

Fig. 1. The recorded Inland habitats of *Plantago maritima* (black circles and triangles), and the Coastal habitats sampled (hatched circles).

II. North Uist (Hebrides):

PMN 23, coll. 10. ix. 28 from a cattle-grazed pasture on the shores of a sea loch near Lochmaddy by the late Dr A. R. Wilson. (1930.)

PMN 25, coll. 12. ix. 28 from a cattle- and sheep-grazed pasture on the shores of a sea loch near Lochmaddy, by the late Dr A. R. Wilson. (1930.)

C. *Coastal.*

(*a*) Waterlogged mud:

PMN 20, coll. 8. x. 28, Solway Firth (Dumfriesshire). (1930.)
PMN 32, coll. 17. ix. 29, Aberlady (East Lothian), sheep-grazed. (1931.)
PMN 33, coll. 7. ix. 29, Ellon (Aberdeenshire), sheep-grazed. (1931.)
PMN 34, coll. 8. ix. 29, Culbin (Elgin), sheep-grazed. (1931.)
PMN 45, coll. 21. viii. 30, Brancaster (Norfolk). (1932.)

(*b*) Salt marsh:

PMN 19, coll. 8. ix. 28, Holy Loch (Argyllshire). (1931.)
PMN 35, coll. 10. ix. 29, Loch Leven (Argyllshire). (1931.)
PMN 36, coll. 11. ix. 29, Loch Feochan (Argyllshire). (1931.)
PMN 46, coll. 21. viii. 30, Blakeney Point (Norfolk). (1932.)
PMN 67, coll. 26. viii. 31, Aberlady (East Lothian). (1933.)

(*c*) Drained Mud:

PMN 9, coll. 20. ix. 27, Aberystwyth (Cardiganshire). (1929.)
PMN 37, coll. 9. ix. 29, Beauly Firth (Inverness-shire.) (1931.)
PMN 54, coll. 27. ix. 30, Dunbar (East Lothian). (1935.)

(*d*) Grass meadows above highest tide mark:

PMN 78, coll. viii. 32, Southampton (Hampshire), by Dr D. Clouston. (1934.)
PMN 80, coll. viii. 32, Brighton (Sussex), by Dr D. Clouston. (1934.)
PMN 87, coll. 18. ix. 33, Wigtown (Wigtownshire). (1935.)
PMN 91, coll. 8. viii. 34, Salcott (Essex), by Dr V. McM. Davey. (1936.)

(*e*) Exposed cliff summit:

PMN 93, coll. 18. ix. 33, Mull of Galloway (Wigtownshire) (plants). (1936.)

The following records provide an additional list of inland habitats for *P. maritima*:

Scotland.

ABERDEEN and BANFFSHIRE. *Rivers Deveron and Isla region:* Keith (Craib, 1912); Grange (Craib); Rothiemay (Craib); Marnoch (Craib); Drumblade (Dickie, 1860); Forgue (Dickie); Turriff (Trail 1902); Alvah (Trail). *River Ythan:* Auchterless (Trail); Fyvie (Trail); Methlick (Trail); Tarves (Trail); Ellon (Trail); *N. and S. Ugie:* New Deer (Trail); Old Deer (Trail); Longside (Trail); Strichen (Trail). *Rivers Don and Urie:* Tullynessle (Dickie); Alford (Murray, 1836); Clatt (Murray); Rayne (Murray); Inverurie (Murray). *River Dee:* Lochnagar (Roy); E. of Morven and Ballater, below 2000 ft. (Trail MS. 1906); Glen Tanner (Trail MS.); Aboyne (Trail MS.); Birse (Trail MS.); Coull (Trail MS.); Kincardine O'Neil (Trail MS.); Hill of Fair, Banchory (Dickie). *River Spey:* Rothes (Trail MS.); Boharm (Craib). *River Desk:* Deskford (Craib). *River Boyne:* Ordiquhill (Craib). ANGUS. Glen Esk (Murray); Glen Clova, 1853, Balfour (Anonymous, 1902); Glen Isla, 1837, Balfour, Herb. Bot. Gard. Edinb.; Airlie (Gardiner, 1848). DUMBARTONSHIRE. Ben Vorlich, 1866, Balfour, Herb. Bot. Gard. Edinb. INVERNESS-SHIRE. Ben Nevis, 1876, Sadler, Herb. Bot. Gard. Edinb. PERTHSHIRE. Loch Rannoch, 1881, Evans, Herb. Bot. Gard. Edinb.; Meall Ghaordie, 1870, Balfour (Anonymous, 1902);

Cam Craig, Loch Earn, 1837, Balfour, Herb. Bot. Gard. Edinb.; Breadalbane, 2600 ft. (White, 1898). ROSS-SHIRE. Achilty, 1868, White, Herb. Bot. Gard. Edinb.; Slioch (Druce, 1929); Benula, Cannich, Adam. SUTHERLANDSHIRE. Ben Hope, 2600 ft. (Melvill, 1889).

England.

CORNWALL. St Day (Davey, 1902); Bodmin (Davey); St Neot (Davey); Launceston (Davey). CUMBERLAND. Moota Hill, between Aspatria and Cockermouth (Hodgson, 1898); Head of Fusedale (Baker, 1885). YORKSHIRE. Cronkleyfell, 1790 ft. (Baker, 1863); High Force, 1730 ft. (Baker, 1863); Winch Bridge, 1836, Herb. Bot. Gard. Edinb.; Askrigg, between there and Carperby, Good (written communication); Seamerdale (Baker, 1863); Arncliffe, between there and Kilnsey (Bains, 1840); Grassington, between there and Kilnsey (Miall, 1862); Settle (Lees, 1888). DERBYSHIRE. Alfreton, 1804, Backhouse, Herb. Bot. Gard. Edinb.

Wales.

CARNARVONSHIRE. Snowdon, 2600 ft. (Williams, 1912, p. 360). Crags of Glyder Faur, 1865. Backhouse, Herb. Bot. Gard. Edinb.

II. THE EXAMINATION OF CHARACTERS

Mean values

The methods of sampling habitat populations, of growing the samples in the experimental garden and of assessing their character values have already been dealt with in the paper previously mentioned (Gregor *et al.* 1936). The samples, according to their source, have been grouped in Table I into the three primary habitat categories—Inland, Island and Coastal; the samples from the Coastal areas are further subdivided into five ecologically distinctive habitat groups thus: (*a*) waterlogged mud, (*b*) typical salt marsh, (*c*) drained mud, (*d*) grassland above highest tide mark, and (*e*) exposed cliff summit. The individual sample mean values and their standard errors, calculated from 100 or more observations, are presented for each character studied and, in addition, a general mean is given for the Inland and Island populations and for each subdivision of the Coastal samples. The characters have been previously defined elsewhere (Gregor *et al.* 1936, p. 329) with the exception of scape volume, which is an approximate value in litres for plant size, calculated from the formula $\pi \left(\frac{scape\ spread}{2}\right)^2 \times$ scape height.

From a perusal of the mean figures for the Coastal categories (*a*), (*b*), (*c*) and (*d*) it will be noticed that for the characters scape spread, scape height, scape length, scape thickness, spike length, scape volume, leaf length, leaf breadth, leaf spread, leaf height and seed length there is a progressive increase in size from habitat (*a*) to habitat (*d*), while for the character spike density there is a progressive decrease in density in the same direction. The differences between the values for

TABLE I. *Mean values*

Habitats	Samples PMN	Habit grade	Scape spread/ height	Scape spread in.	Scape height in.	Scape length cm.	Scape thickness mm.	Spike length cm.	Scape volume litres
Inland: I	17	2·00 ± 0·102	1·56 ± 0·030	18·3 ± 0·37	11·9 ± 0·28	30·2 ± 0·61	1·55 ± 0·030	6·91 ± 0·163	3·45 ± 0·177
	18	2·75 ± 0·042	1·26 ± 0·016	17·8 ± 0·22	14·3 ± 0·17	36·3 ± 0·37	1·82 ± 0·015	9·74 ± 0·120	3·85 ± 0·131
II	16	1·76 ± 0·076	1·54 ± 0·022	18·4 ± 0·24	12·2 ± 0·20	32·0 ± 0·44	1·42 ± 0·017	6·85 ± 0·129	3·37 ± 0·122
	38	2·26 ± 0·084	1·43 ± 0·026	16·0 ± 0·32	11·5 ± 0·30	31·1 ± 0·69	1·44 ± 0·024	7·89 ± 0·206	2·58 ± 0·169
	39	2·32 ± 0·097	1·62 ± 0·039	18·8 ± 0·37	12·1 ± 0·30	33·7 ± 0·62	1·35 ± 0·017	7·47 ± 0·169	3·69 ± 0·219
III	40	2·42 ± 0·074	1·45 ± 0·023	19·9 ± 0·33	14·0 ± 0·21	36·3 ± 0·45	1·66 ± 0·023	9·35 ± 0·172	4·55 ± 0·186
	55	2·63 ± 0·081	1·37 ± 0·027	18·0 ± 0·28	13·4 ± 0·24	35·6 ± 0·49	1·46 ± 0·021	8·85 ± 0·169	3·52 ± 0·139
	21	2·81 ± 0·065	1·22 ± 0·018	18·7 ± 0·25	15·6 ± 0·23	39·3 ± 0·51	1·87 ± 0·022	9·72 ± 0·168	4·47 ± 0·158
	90	2·85 ± 0·063	1·10 ± 0·018	16·2 ± 0·28	14·8 ± 0·21	38·9 ± 0·52	1·84 ± 0·019	10·17 ± 0·194	3·21 ± 0·146
Mean		2·42	1·39	18·01	13·30	34·82	1·60	8·55	3·63
Island: I	47	1·21 ± 0·056	2·27 ± 0·092	8·5 ± 0·22	4·1 ± 0·14	14·9 ± 0·40	1·30 ± 0·025	3·82 ± 0·148	0·29 ± 0·021
	48	1·66 ± 0·080	2·01 ± 0·078	13·5 ± 0·39	7·5 ± 0·30	23·8 ± 0·65	1·40 ± 0·026	6·56 ± 0·226	1·34 ± 0·111
	49	2·71 ± 0·074	1·15 ± 0·021	14·4 ± 0·26	12·7 ± 0·21	33·8 ± 0·45	1·83 ± 0·025	10·15 ± 0·178	2·20 ± 0·098
II	25	1·89 ± 0·066	1·89 ± 0·069	16·0 ± 0·26	9·1 ± 0·21	26·7 ± 0·40	1·59 ± 0·021	6·94 ± 0·148	1·95 ± 0·091
	23	1·91 ± 0·049	1·58 ± 0·024	16·2 ± 0·25	10·6 ± 0·20	28·7 ± 0·44	1·68 ± 0·020	8·17 ± 0·135	2·39 ± 0·111
Mean		1·88	1·78	13·72	8·80	25·58	1·56	7·13	1·63
Coastal: (*a*)	32	1·29 ± 0·054	1·98 ± 0·055	13·1 ± 0·31	7·1 ± 0·23	22·1 ± 0·54	1·72 ± 0·029	6·62 ± 0·181	1·10 ± 0·069
	33	1·56 ± 0·072	1·68 ± 0·038	14·5 ± 0·31	8·9 ± 0·23	27·1 ± 0·52	1·96 ± 0·027	8·00 ± 0·162	1·64 ± 0·096
	34	1·69 ± 0·083	1·57 ± 0·039	12·1 ± 0·36	7·9 ± 0·23	22·7 ± 0·54	1·76 ± 0·036	6·46 ± 0·195	1·14 ± 0·094
	20	1·80 ± 0·064	1·55 ± 0·029	16·7 ± 0·20	11·4 ± 0·21	32·3 ± 0·46	2·07 ± 0·019	10·11 ± 0·166	2·68 ± 0·102
	45	1·97 ± 0·083	1·41 ± 0·033	16·0 ± 0·42	11·7 ± 0·32	35·7 ± 0·75	1·81 ± 0·033	11·44 ± 0·290	2·76 ± 0·189
Mean		1·66	1·64	14·48	9·40	27·98	1·864	8·53	1·86
(*b*)	19	2·00 ± 0·095	1·46 ± 0·039	15·4 ± 0·29	11·1 ± 0·33	31·8 ± 0·66	2·11 ± 0·029	9·25 ± 0·202	2·34 ± 0·136
	35	2·09 ± 0·076	1·31 ± 0·033	15·0 ± 0·36	10·2 ± 0·24	27·8 ± 0·48	1·74 ± 0·030	7·19 ± 0·175	2·02 ± 0·133
	46	2·22 ± 0·066	1·37 ± 0·019	17·7 ± 0·40	13·0 ± 0·28	36·5 ± 0·63	1·83 ± 0·026	10·62 ± 0·242	3·64 ± 0·218
	36	2·25 ± 0·063	1·42 ± 0·029	15·1 ± 0·32	11·1 ± 0·22	31·4 ± 0·58	1·78 ± 0·020	9·01 ± 0·150	2·23 ± 0·127
	67	2·59 ± 0·071	1·31 ± 0·024	18·4 ± 0·34	14·3 ± 0·28	38·7 ± 0·58	2·03 ± 0·027	10·75 ± 0·210	4·01 ± 0·219
Mean		2·23	1·41	16·32	11·94	33·24	1·898	9·36	2·85
(*c*)	9	2·87 ± 0·064	1·00 ± 0·017	17·6 ± 0·30	17·8 ± 0·23	44·5 ± 0·51	2·23 ± 0·027	9·82 ± 0·149	4·64 ± 0·195
	37	2·97 ± 0·057	0·90 ± 0·017	15·6 ± 0·38	17·4 ± 0·30	45·3 ± 0·63	2·25 ± 0·028	11·44 ± 0·265	3·67 ± 0·209
	54	3·33 ± 0·056	0·81 ± 0·013	16·5 ± 0·29	20·3 ± 0·22	49·3 ± 0·52	2·70 ± 0·030	12·60 ± 0·217	4·53 ± 0·204
Mean		3·06	0·903	16·57	18·50	46·37	2·39	11·29	4·28
(*d*)	91	2·78 ± 0·075	1·08 ± 0·018	18·7 ± 0·30	17·7 ± 0·29	45·4 ± 0·66	2·11 ± 0·025	10·43 ± 0·199	5·14 ± 0·203
	80	2·99 ± 0·033	1·13 ± 0·017	23·3 ± 0·39	20·9 ± 0·30	52·5 ± 0·67	2·51 ± 0·038	13·96 ± 0·278	9·42 ± 0·342
	78	3·05 ± —	1·10 ± 0·015	24·8 ± 0·46	22·4 ± 0·42	53·8 ± 1·10	2·33 ± 0·043	13·60 ± 0·375	11·18 ± 0·570
	87	3·23 ± 0·061	0·84 ± 0·015	17·5 ± 0·32	21·1 ± 0·24	50·6 ± 0·54	2·64 ± 0·026	12·64 ± 0·199	5·32 ± 0·217
Mean		3·01	1·04	21·08	20·53	50·58	2·40	12·66	7·77
(*e*)	93	1·41 ± 0·059	2·33 ± 0·058	14·3 ± 0·24	6·4 ± 0·16	19·4 ± 0·35	1·22 ± 0·017	4·13 ± 0·093	1·10 ± 0·059

Habitats	Samples PMN	Leaf length cm.	Leaf breadth mm.	Leaf thickness mm.	Leaf spread in.	Leaf height in.	Leaf spread/ height	Spike density	Bract length mm.
Inland: I	17	23·1 ± 0·49	5·70 ± 0·201	1·04 ± 0·016	9·1 ± 0·31	3·12 ± 0·115	2·98 ± 0·086	—	—
	18	27·7 ± 0·38	6·88 ± 0·125	1·06 ± 0·012	9·4 ± 0·16	3·69 ± 0·081	2·80 ± 0·042	—	—
II	16	22·7 ± 0·37	4·97 ± 0·144	1·00 ± 0·013	9·2 ± 0·21	3·00 ± 0·085	3·20 ± 0·078	—	—
	38	19·7 ± 0·59	4·97 ± 0·181	0·82 ± 0·015	11·0 ± 0·30	2·58 ± 0·078	4·47 ± 0·116	13·4 ± 0·20	2·48 ± 0·036
	39	21·4 ± 0·57	4·87 ± 0·155	0·83 ± 0·012	10·7 ± 0·30	2·75 ± 0·090	4·12 ± 0·115	13·1 ± 0·21	2·27 ± 0·031
III	40	25·1 ± 0·43	6·60 ± 0·188	0·80 ± 0·012	12·0 ± 0·26	4·38 ± 0·130	2·86 ± 0·064	14·4 ± 0·21	2·78 ± 0·033
	55	21·1 ± 0·39	5·38 ± 0·136	0·82 ± 0·011	11·2 ± 0·20	3·28 ± 0·081	3·55 ± 0·072	15·0 ± 0·21	—
	21	27·3 ± 0·37	7·33 ± 0·159	0·95 ± 0·012	14·8 ± 0·24	4·73 ± 0·134	3·34 ± 0·087	13·8 ± 0·24	2·80 ± 0·039
	90	21·8 ± 0·33	5·80 ± 0·162	0·79 ± 0·010	11·6 ± 0·22	3·74 ± 0·093	3·20 ± 0·059	14·1 ± 0·19	2·65 ± 0·032
Mean		23·32	5·83	0·901	11·00	3·47	3·39	13·97	2·60
Island: I	47	11·5 ± 0·41	3·87 ± 0·134	0·98 ± 0·014	3·48 ± 0·142	1·04 ± —	3·36 ± 0·135	12·6 ± 0·23	2·29 ± 0·043
	48	18·2 ± 0·50	4·94 ± 0·183	0·90 ± 0·013	6·18 ± 0·238	1·70 ± —	3·85 ± 0·118	14·1 ± 0·27	2·58 ± 0·061
	49	22·2 ± 0·45	6·77 ± 0·182	0·98 ± 0·012	7·71 ± 0·249	2·46 ± 0·099	3·39 ± 0·110	14·9 ± 0·27	2·93 ± 0·050
II	25	20·4 ± 0·45	5·33 ± 0·155	1·18 ± 0·015	8·99 ± 0·227	2·80 ± 0·093	3·51 ± 0·105	—	—
	23	23·2 ± 0·42	4·68 ± 0·122	1·10 ± 0·013	9·23 ± 0·216	2·82 ± 0·079	3·57 ± 0·075	—	—
Mean		19·10	5·12	1·03	7·12	2·16	3·54	13·87	2·60
Coastal: (*a*)	32	15·8 ± 0·52	4·14 ± 0·181	1·02 ± 0·018	6·1 ± 0·22	1·81 ± 0·073	3·51 ± 0·098	13·9 ± 0·21	2·48 ± 0·030
	33	20·1 ± 0·53	5·90 ± 0·175	1·24 ± 0·034	7·0 ± 0·25	2·02 ± 0·063	3·03 ± 0·129	12·9 ± 0·18	2·74 ± 0·032
	34	16·6 ± 0·56	3·89 ± 0·168	1·18 ± 0·018	6·6 ± 0·26	1·71 ± 0·077	4·09 ± 0·141	—	—
	20	22·1 ± 0·35	5·43 ± 0·118	1·18 ± 0·012	9·7 ± 0·18	2·75 ± 0·059	3·81 ± 0·065	—	—
	45	21·0 ± 0·55	6·40 ± 0·232	0·97 ± 0·017	8·4 ± 0·36	2·24 ± 0·102	3·93 ± 0·119	12·0 ± 0·19	2·69 ± 0·050
Mean		19·12	5·16	1·12	7·56	2·11	3·67	12·93	2·64
(*b*)	19	23·3 ± 0·59	5·44 ± 0·199	1·25 ± 0·018	9·1 ± 0·30	2·26 ± 0·088	4·30 ± 0·157	12·0 ± 0·14	2·55 ± 0·034
	35	24·0 ± 0·58	6·47 ± 0·242	1·19 ± 0·020	8·3 ± 0·26	2·20 ± 0·094	4·13 ± 0·141	—	—
	46	23·3 ± 0·54	7·41 ± 0·213	1·08 ± 0·016	10·3 ± 0·36	2·75 ± 0·123	4·06 ± 0·116	11·6 ± 0·26	2·79 ± 0·067
	36	22·1 ± 0·44	5·64 ± 0·145	1·14 ± 0·015	7·7 ± 0·21	2·07 ± 0·069	4·03 ± 0·109	12·7 ± 0·14	2·33 ± 0·032
	67	24·8 ± 0·48	7·18 ± 0·186	0·98 ± 0·017	12·1 ± 0·31	2·93 ± 0·088	4·32 ± 0·106	13·3 ± 0·19	2·40 ± 0·031
Mean		23·50	6·43	1·13	9·50	2·44	4·17	12·40	2·52
(*c*)	9	26·9 ± 0·37	9·38 ± 0·161	1·20 ± 0·020	14·4 ± 0·28	4·57 ± 0·165	3·52 ± 0·101	—	—
	37	34·5 ± 0·56	9·16 ± 0·210	1·33 ± 0·018	13·0 ± 0·29	4·11 ± 0·136	3·37 ± 0·089	11·1 ± 0·15	2·87 ± 0·039
	54	36·1 ± 0·47	12·20 ± 0·247	1·17 ± 0·012	18·4 ± 0·24	5·24 ± 0·136	3·68 ± 0·078	13·4 ± 0·22	3·15 ± 0·036
Mean		32·50	10·25	1·23	15·27	4·64	3·52	12·25	3·01
(*d*)	91	30·9 ± 0·54	9·65 ± 0·156	1·04 ± 0·017	15·7 ± 0·28	5·52 ± 0·155	2·97 ± 0·068	11·6 ± 0·13	2·82 ± 0·028
	80	35·5 ± 0·64	11·48 ± 0·270	1·06 ± 0·014	18·9 ± 0·43	6·63 ± 0·243	3·13 ± 0·086	12·0 ± 0·18	3·30 ± 0·046
	78	32·2 ± 0·63	11·74 ± 0·412	1·00 ± 0·016	19·5 ± 0·45	7·08 ± 0·248	2·82 ± 0·069	12·3 ± 0·25	2·97 ± 0·063
	87	32·9 ± 0·48	11·53 ± 0·225	1·07 ± 0·011	15·1 ± 0·28	4·84 ± 0·130	3·27 ± 0·066	13·0 ± 0·17	2·83 ± 0·033
Mean		32·88	11·10	1·04	17·30	6·02	3·05	12·23	2·98
(*e*)	93	14·9 ± 0·29	5·80 ± 0·162	0·77 ± 0·011	7·21 ± 0·19	2·90 ± 0·069	2·48 ± 0·033	15·1 ± 0·20	1·99 ± 0·024

Table I (continued)

Habitats	Samples PMN	Bract breadth mm.	Bract index	Sepal length mm.	Sepal breadth mm.	Sepal index	Seed length mm.	Seed breadth mm.	Seed index
Inland: I	17	—	—	—	—	—	—	—	—
	18	—	—	—	—	—	—	—	—
II	16	—	—	—	—	—	—	—	—
	38	1·15 ± 0·013	2·17 ± 0·026	2·43 ± 0·023	1·04 ± 0·010	2·34 ± 0·027	2·40 ± 0·015	0·99 ± 0·006	2·42 ± 0·015
	39	1·11 ± 0·012	2·06 ± 0·026	2·37 ± 0·020	1·02 ± 0·011	2·33 ± 0·027	2·35 ± 0·015	1·03 ± 0·007	2·28 ± 0·016
III	40	1·32 ± 0·015	2·14 ± 0·023	2·51 ± 0·020	1·12 ± 0·009	2·25 ± 0·021	2·38 ± 0·018	1·02 ± 0·007	2·35 ± 0·016
	55	—	—	—	—	—	2·30 ± 0·015	0·99 ± 0·006	2·33 ± 0·015
	21	1·24 ± 0·013	2·25 ± 0·030	2·47 ± 0·019	1·11 ± 0·013	2·25 ± 0·025	2·43 ± 0·018	1·01 ± 0·008	2·43 ± 0·016
	90	1·21 ± 0·011	2·19 ± 0·022	2·35 ± 0·014	1·07 ± 0·008	2·21 ± 0·018	2·40 ± 0·011	0·99 ± 0·006	2·43 ± 0·016
Mean		1·21	2·16	2·43	1·07	2·28	2·38	1·01	2·37
Island: I	47	1·37 ± 0·020	1·68 ± 0·030	2·46 ± 0·026	—	—	2·57 ± 0·016	1·05 ± 0·007	2·46 ± 0·016
	48	1·24 ± 0·024	2·08 ± 0·039	2·49 ± 0·049	—	—	2·13 ± 0·019	0·94 ± 0·006	2·27 ± 0·020
	49	1·37 ± 0·021	2·14 ± 0·032	2·78 ± 0·030	—	—	2·37 ± 0·012	1·02 ± 0·005	2·33 ± 0·014
II	25	—	—	—	—	—	—	—	—
	23	—	—	—	—	—	—	—	—
Mean		1·33	1·97	2·58	—	—	2·36	1·00	2·35
Coastal: (*a*)	32	1·33 ± 0·012	1·87 ± 0·023	—	—	—	2·37 ± 0·017	0·99 ± 0·006	2·41 ± 0·014
	33	1·55 ± 0·013	1·78 ± 0·015	—	—	—	2·62 ± 0·025	1·05 ± 0·005	2·51 ± 0·013
	34	—	—	—	—	—	2·53 ± 0·015	1·06 ± 0·006	2·39 ± 0·013
	20	—	—	—	—	—	—	—	—
	45	1·32 ± 0·016	2·02 ± 0·026	2·62 ± 0·027	—	—	—	—	—
Mean		1·40	1·89	2·62	—	—	2·51	1·03	2·44
(*b*)	19	1·41 ± 0·015	1·83 ± 0·018	—	—	—	2·59 ± 0·018	1·05 ± 0·007	2·47 ± 0·014
	35	—	—	—	—	—	2·68 ± 0·016	1·10 ± 0·007	2·44 ± 0·015
	46	1·40 ± 0·025	2·00 ± 0·030	2·72 ± 0·032	—	—	2·75 ± 0·018	1·00 ± 0·006	2·76 ± 0·017
	36	1·44 ± 0·014	1·62 ± 0·017	—	—	—	2·73 ± 0·017	1·07 ± 0·007	2·56 ± 0·015
	67	1·32 ± 0·014	1·83 ± 0·019	2·45 ± 0·012	1·18 ± 0·012	2·10 ± 0·022	2·84 ± 0·017	1·10 ± 0·007	2·59 ± 0·018
Mean		1·39	1·82	2·59	1·18	2·10	2·72	1·06	2·56
(*c*)	9	—	—	—	—	—	—	—	—
	37	1·69 ± 0·019	1·70 ± 0·020	—	—	—	2·82 ± 0·017	1·13 ± 0·007	2·51 ± 0·014
	54	1·48 ± 0·011	2·14 ± 0·022	2·61 ± 0·018	1·34 ± 0·010	1·95 ± 0·018	2·97 ± 0·023	1·11 ± 0·008	2·69 ± 0·018
Mean		1·59	1·92	2·61	1·34	1·95	2·90	1·12	2·60
(*d*)	91	1·37 ± 0·013	2·07 ± 0·019	2·57 ± 0·020	1·32 ± 0·012	1·96 ± 0·020	2·96 ± 0·015	1·10 ± 0·007	2·71 ± 0·016
	80	1·56 ± 0·016	2·11 ± 0·022	2·81 ± 0·026	1·35 ± 0·010	2·08 ± 0·019	2·98 ± 0·028	1·08 ± 0·008	2·79 ± 0·023
	78	1·52 ± 0·026	1·97 ± 0·025	2·56 ± 0·035	1·25 ± 0·019	2·05 ± 0·030	3·01 ± 0·028	1·07 ± 0·010	2·78 ± 0·020
	87	1·38 ± 0·012	2·07 ± 0·022	2·47 ± 0·015	1·28 ± 0·010	1·94 ± 0·016	3·01 ± 0·022	1·10 ± 0·008	2·73 ± 0·015
Mean		1·46	2·06	2·60	1·30	2·01	2·99	1·09	2·75
(*e*)	93	1·19 ± 0·013	1·69 ± 0·021	2·17 ± 0·020	1·09 ± 0·010	1·99 ± 0·019	2·03 ± 0·011	0·92 ± 0·006	2·22 ± 0·016

Habitats	Samples PMN	Anther length mm.	Anther tip length mm.	Bract length/ sepal length	Scape length/ spike length	Scape length/ leaf length	Flowering grade
Inland: I	17	—	—	—	4·44 ± 0·086	1·33 ± 0·031	—
	18	—	—	—	3·78 ± 0·064	1·35 ± 0·027	—
II	16	—	—	—	4·78 ± 0·079	1·42 ± 0·025	—
	38	1·89 ± 0·019	0·284 ± 0·006	1·02 ± 0·010	4·03 ± 0·064	1·65 ± 0·027	7·18 ± 0·165
	39	1·80 ± 0·018	0·271 ± 0·006	0·96 ± 0·011	4·60 ± 0·074	1·62 ± 0·028	7·45 ± 0·177
III	40	2·06 ± 0·014	0·299 ± 0·006	1·11 ± 0·011	3·97 ± 0·063	1·47 ± 0·022	5·02 ± 0·136
	55	—	—	—	4·12 ± 0·066	1·72 ± 0·024	4·13 ± 0·158
	21	2·10 ± 0·021	0·282 ± 0·005	1·15 ± 0·013	4·13 ± 0·063	1·46 ± 0·018	3·77 ± 0·123
	90	1·98 ± 0·018	0·333 ± 0·006	1·14 ± 0·012	3·91 ± 0·057	1·82 ± 0·029	2·94 ± 0·124
Mean		1·97	0·294	1·08	4·20	1·54	5·08
Island: I	47	2·01 ± 0·026	0·249 ± 0·007	0·93 ± 0·016	4·15 ± 0·107	1·39 ± 0·040	6·85 ± 0·231
	48	2·04 ± 0·032	0·250 ± 0·006	1·04 ± 0·016	3·82 ± 0·099	1·43 ± 0·030	3·97 ± 0·107
	49	2·23 ± 0·029	0·243 ± 0·008	1·05 ± 0·016	3·30 ± 0·047	1·56 ± 0·024	3·30 ± 0·089
	25	—	—	—	3·93 ± 0·067	1·35 ± 0·029	—
II	23	—	—	—	3·59 ± 0·061	1·27 ± 0·023	—
Mean		2·09	0·247	1·01	3·76	1·40	4·71
Coastal: (*a*)	32	2·19 ± 0·033	0·242 ± 0·009	—	3·39 ± 0·051	1·50 ± 0·032	2·43 ± 0·146
	33	2·36 ± 0·024	0·269 ± 0·009	—	3·42 ± 0·050	1·48 ± 0·027	1·96 ± 0·106
	34	2·16 ± 0·031	0·267 ± 0·009	—	3·63 ± 0·067	1·44 ± 0·030	3·28 ± 0·175
	20	—	—	—	3·31 ± 0·050	1·48 ± 0·029	—
	45	2·36 ± 0·027	0·270 ± 0·008	1·04 ± 0·016	3·20 ± 0·053	1·73 ± 0·025	2·88 ± 0·126
Mean		2·27	0·262	1·04	3·39	1·53	2·64
(*b*)	19	2·19 ± 0·038	0·243 ± 0·010	—	3·51 ± 0·067	1·53 ± 0·023	2·09 ± 0·114
	35	2·04 ± 0·027	0·291 ± 0·007	—	3·91 ± 0·067	1·18 ± 0·023	5·52 ± 0·187
	46	2·32 ± 0·027	0·270 ± 0·008	1·03 ± 0·018	3·46 ± 0·050	1·61 ± 0·032	6·60 ± 0·189
	36	2·11 ± 0·028	0·255 ± 0·009	—	3·43 ± 0·039	1·44 ± 0·020	3·32 ± 0·101
	67	2·02 ± 0·019	0·285 ± 0·006	0·97 ± 0·010	3·67 ± 0·059	1·59 ± 0·025	4·17 ± 0·204
Mean		2·14	0·269	1·00	3·60	1·47	4·34
(*c*)	9	—	—	—	4·60 ± 0·083	1·66 ± 0·022	—
	37	2·58 ± 0·030	0·279 ± 0·008	—	3·84 ± 0·078	1·34 ± 0·018	1·91 ± 0·098
	54	2·18 ± 0·018	0·300 ± 0·006	1·22 ± 0·012	3·95 ± 0·057	1·38 ± 0·015	1·23 ± —
Mean		2·38	0·290	1·22	4·13	1·46	1·57
(*d*)	91	2·27 ± 0·017	0·252 ± 0·006	1·10 ± 0·009	4·44 ± 0·066	1·51 ± 0·025	7·64 ± 0·102
	80	2·32 ± 0·020	0·279 ± 0·007	1·18 ± 0·013	3·83 ± 0·069	1·50 ± 0·028	6·75 ± 0·161
	78	2·31 ± 0·022	0·248 ± 0·007	1·17 ± 0·019	4·04 ± 0·104	1·70 ± 0·028	7·02 ± 0·260
	87	2·22 ± 0·014	0·287 ± 0·005	1·15 ± 0·011	4·08 ± 0·060	1·35 ± 0·017	1·01 ± —
Mean		2·28	0·267	1·15	4·10	1·57	5·61
(*e*)	93	2·00 ± 0·017	0·234 ± 0·006	0·92 ± 0·010	4·81 ± 0·079	1·33 ± 0·021	7·89 ± 0·153

the characters scape thickness, spike length, leaf height, and spike density however, do not reach the 0·05 level of significance (Fisher, 1932, Table of *t*) and therefore these characters cannot be said to exhibit any significant effect of habitat conditions. If the habitat category (*d*) be disregarded, then in addition to the above the characters habit grade, leaf thickness, anther tip length and the ratio scape length : spike length show mean values increasing towards habitat (*c*), while the values for scape spread : height and scape length : leaf length become progressively less, i.e. the samples tend to become more erect and the leaves to be longer in proportion to the scapes under conditions prevailing in (*c*) habitats. The differences between the means for the characters leaf thickness, anther tip length and the ratio scape length : leaf length are, however, not significant. The characters leaf spread : height, bract length, bract breadth, bract index and anther length fail to follow a sequence.

Now if the characters which exhibit no significant sequence or lack sequence altogether are disregarded then the following remain: habit grade, scape spread : height, scape spread, scape height, scape length, scape volume, leaf length, leaf breadth, leaf spread, seed length and scape length : spike length. With the exception of the character scape length : spike length, a ratio indicating that the spikes become shorter in proportion to the scape as the scapes increase in length, all these characters represent measurements either of growth habit or size of scapes, seeds and leaves. These data, therefore, lead to the conclusion that while the dimensions of floral parts, e.g. seeds, may be influenced to some extent by general plant size, and therefore indirectly by habitat conditions, it is primarily the habit and size of the scapes, and the leaf dimensions which are affected by the environment. It would indeed be difficult to establish a geographical sequence within the sampled area in the distribution of such character owing to this marked influence of the habitat factors. For example the differences in growth-habit and size between the samples PMN 32 and 67 from distinctive but adjacent habitats in East Lothian are considerably greater than those occurring between the samples PMN 36 and 46 collected from ecologically similar habitats as far apart as Argyllshire and Norfolk respectively.

The fractionation of plantain populations into localized habitat races by environment is repeated in Lewis, one of the Western Islands. Only three samples have been examined from this island but they are representative of different types of habitat; PMN 47 belongs to the (*a*) habitat category, PMN 48 was collected from a

partially populated cliff habitat, while PMN 49 was collected from a coastal meadow in many respects equivalent to the (*d*) category of the mainland. If habit grade and scape length, leaf length and scape volume are again taken as the respective criteria of habit of growth and size, it will be seen from Table I that there is, as previously, a significant progressive increase in the erectness and size of the sample constituents from the waterlogged to the meadow habitat. In this instance, however, the size increase is also noticeable in the lengths of both bracts and sepals, although the difference between PMN 47 and 48 for the latter character is not significant.

It is plain from the above examples that dwarf growth accompanies a high water table; but high winds and the influence of the grazing animal produce similar effects. For example, PMN 93, from an exposed cliff habitat subject to sheep grazing, resembled in many respects samples PMN 47, 32, 33 and 34 collected from waterlogged mud flats. In the natural habitats the parallelism was even more pronounced than in the experimental garden because in the wild the leaves of all these populations were short (1–4 cm. in length) and more or less cylindrical. In culture, however, PMN 93 exhibited a more cushion-like appearance than did the mud-flat samples, largely owing to the crowding together of the numerous short leaves and scapes. Of all the population samples examined from Britain PMN 93 had the highest value for the ratio scape spread : height, $2{\cdot}33 \pm 0{\cdot}058$, the next highest in the series being the Island population PMN 47 with $2{\cdot}27 \pm 0{\cdot}092$; the difference, however, is not significant. In PMN 93 the size characters scape length, leaf length and scape volume have mean values which fall between the values for PMN 47 and 32 both of which samples are characterized by the small size of their component plants. Decumbent growth-habit and dwarfness are therefore not peculiar to water-logged habitats.

In the Inland samples, as far as the growth-habit and the size of the vegetative organs are concerned, the mean values fall within the Coastal range. A few of the mean values for the floral characters (bract breadth, bract index, sepal breadth, sepal index, seed length and seed index), however, fall beyond the Coastal range, but only in the case of bract index (PMN 21), sepal breadth (PMN 39) and sepal index (PMN 38, 39, 40, 55, 21) are these deviations significant. Of the Inland characters sepal index alone has mean values which do not overlap those of the Coastal series. Unfortunately the data for this character are lacking for several populations, but in so far as the figures are available the Inland samples are, on the average, shown

to possess sepals narrower in proportion to their length than do the Coastal samples.

It might have been expected that as the altitude of the Inland habitats increased, the size of the population components would decrease, but it has not been possible to establish any regular sequence in this direction. For example PMN 17, collected from a waterlogged habitat at an elevation of 500 ft. is seen to have size characters considerably smaller than those of PMN 18 from a habitat at 800 ft. However PMN 28, a numerically small sample from a habitat at an altitude 1200 ft. higher than the habitat of PMN 18 and 1500 ft. higher than the habitat of PMN 17, but still on the same stream, was found to be appreciably smaller than PMN 17. Under these circumstances therefore, the tendency for a vertical diminution of size characters is largely masked by the diversity of the habitat conditions at similar altitudinal levels.

In determining the relative times of flowering of the samples a scale of flowering stages was employed, and each plant in a sample was classed as belonging to one of eleven grades, the earliness increasing from grade I to grade II. If, in order to eliminate possible seasonal effects, the values for the year 1931 are compared by themselves it will be observed that some of the samples differ significantly from each other, e.g. the difference between the values for PMN 32 and 35 is thirteen times greater than its error. While no connexion between flowering time and latitude can be established, the averages of the mean values for the different habitat categories suggest that the samples from the (*a*) and (*c*) categories tend to be later flowering then those from (*b*) and (*d*). Even after the individual samples were adjusted on the basis of the seasonal behaviour of a control sample the same relative order was maintained, the adjusted average values for the habitat categories being: for (*a*), 2·74; (*b*) 4·09; (*c*) 1·68; and for (*d*) 5·88. However, in Lewis the sample PMN 47 from an (*a*) habitat flowered significantly earlier than PMN 49 from a (*d*) category, ($D/E_d = 14{\cdot}4$). From a consideration of these data it would seem that local environmental peculiarities play a considerable part in determining the flowering time of these populations.

Range values

While the mean values provide a satisfactory basis for assessing the differences between the genetically impure plantain assemblages, the sample ranges can be usefully employed in detecting the distribution of peculiar variates. It has already been mentioned that the

Inland mean values for the character sepal index do not overlap the corresponding values for the Coastal samples. When, however, the ranges for this character are examined (Table II) it will be seen that continuity in respect of the individual variates is established and that only the variates at the upper end of the range are peculiar to the Inland samples. The same is true for the character bract index.

The Island samples possess variates more dwarf than any found either in the Inland or Coastal samples, as may be seen from the leaf length, scape length, scape height and scape spread : height ranges. That plant size tends to be smaller in the Island samples can be substantiated by reference to the mean values of samples from equivalent habitats on the Mainland. For example the Mainland equivalent of PMN 47 is PMN 32 and of PMN 49 is PMN 80; their respective mean values for scape length are 14·9, 22·1, 33·8 and 52·5 cm., and for leaf length are 11·5, 15·8, 22·2 and 35·5 cm.

The salient feature of the summarized ranges of the Coastal habitat categories is the presence of a high degree of overlapping. Moreover, the extreme variates do not appear to group themselves in any particular geographical order, although for the (*c*) habitat category the largest variates have been found in a southern population and the smallest in a northern one. The sample mean values do not, however, confirm this sequence.

The characters leaf pubescence and leaf spot

Leaf pubescence. The leaves were examined under a 12× lens and the degree of hairiness of the sample components was classified into nine arbitrary grades thus: (1) glabrous, (2) very few scattered hairs on margins, (3) few scattered hairs on margins, (4) a considerable number of hairs on margins, (5) hairs on margins and very few scattered hairs on lower surfaces, (6) hairs on margins and scattered hairs on lower surface, (7) hairs on margins and scattered hairs on upper surface, (8) hairs on margins and scattered hairs on both surfaces, and (9) thinly pubescent.

The percentage number of plants occurring in each grade is summarized in Table III according to the habitats. If the grades of maximum pubescence for the margins, lower surfaces, and both surfaces, respectively grades (4), (6) and (9), are taken as the criteria of greatest pubescence, and the percentage number of variates occurring in these grades calculated, then the Island samples with 55% are the most hairy, the Inland with 34% are the next and the Coastal with 6% are the least pubescent. However, with the exception of

TABLE II. *Character ranges (summary)*

(For full character headings see Table I

Habitat	HbG	ScS:H	ScS in.	ScH in.	ScL cm.	ScTh mm.	SpL cm.	ScV cm.	LfL cm.	LfB mm.	LfTh mm.
Inland	1–5	0·8–3·2	9–29	5–22	18–53	1·0–2·5	4–16	0·6–13·2	12–46	2–13	0·5–1·7
Island	1–5	0·7–7·1	4–26	1–18	5–44	0·7–2·5	1–14	0·1– 8·0	3–39	2–13	0·6–1·5
Coastal:											
Total	1–5	0·5–5·6	4–31	2–27	7–69	0·8–3·5	1–21	0·1–20·4	4–51	1–22	0·5–2·0
Habitat (*a*)	1–4	0·8–3·8	4–24	2–20	7–51	1·0–2·9	2–21	0·1– 8·0	4–35	1–12	0·5–2·0
(*b*)	1–4	0·8–2·9	6–28	3–22	8–53	1·1–2·9	1–17	0·1–12·9	9–41	2–18	0·6–1·7
(*c*)	1–5	0·5–1·6	6–27	8–25	23–64	1·2–3·5	6–20	0·3–12·6	16–50	5–22	0·7–1·9
(*d*)	1–5	0·6–1·5	9–31	11–27	29–69	1·4–3·3	6–20	0·8–20·4	15–51	5–20	0·7–1·4
(*e*)	1–3	1·4–5·6	7–19	2–11	10–27	0·8–1·6	2– 6	0·1– 3·1	9–24	2–10	0·5–1·1

Habitat	LfS in.	LfH in.	LfS:H	SpD	BrL mm.	BrB mm.	BrIx	SepL mm.	SepB mm.
Inland	3–23	1·0–10	1·5–8·7	9–21	1·63–3·56	0·81–1·63	1·5–3·2	1·89–3·00	0·73–1·37
Island	2–15	1·0– 6	1·0–8·0	9–19	1·67–3·99	0·81–1·84	1·2–2·7	1·67–3·26	—
Coastal:									
Total	2–28	1·0–13	1·3–9·0	7–20	1·50–4·16	0·94–2·14	1·1–2·8	1·71–3·39	0·90–1·67
Habitat (*a*)	2–16	1·0– 8	1·6–8·0	8–20	1·71–3·86	0·99–1·93	1·4–2·7	2·19–3·00	—
(*b*)	2–22	1·0–10	1·3–9·0	8–18	1·63–4·16	0·99–1·93	1·1–2·6	1·93–3·39	0·94–1·50
(*c*)	5–23	1·0–11	1·5–8·5	7–18	2·06–4·07	1·24–2·14	1·1–2·8	2·14–3·00	1·03–1·63
(*d*)	8–28	2·5–13	1·8–6·4	9–19	1·97–4·11	1·16–1·71	1·3–2·7	2·06–3·34	0·99–1·67
(*e*)	3–12	1·5– 5	2·0–3·3	9–16	1·50–2·53	0·94–1·54	1·3–2·3	1·71–2·61	0·90–1·37

Habitat	SepIx	SdL mm.	SdB mm.	SdIx	AL mm.	ATL mm.	BrL/SepL	ScL/SpL	ScL/LfL
Inland	1·6–3·2	1·80–2·74	0·81–1·24	1·8–3·0	1·46–2·66	0·13–0·47	0·7–1·5	2·7–7·2	0·9–2·6
Island	—	1·63–3·26	0·81–1·29	1·8–3·1	1·46–2·83	0·09–0·39	0·7–1·4	2·5–8·5	0·7–2·6
Coastal:									
Total	1·5–2·7	1·76–3·59	0·77–1·46	1·8–3·5	1·63–3·09	0·09–0·47	0·7–1·5	1·8–7·4	0·7–2·8
Habitat (*a*)	—	1·89–3·17	0·77–1·33	1·8–3·1	1·76–2·87	0·13–0·43	0·9–1·4	1·8–5·5	0·8–2·8
(*b*)	1·5–2·6	1·97–3·51	0·77–1·37	1·9–3·5	1·63–2·91	0·09–0·47	0·8–1·4	2·4–6·3	0·7–2·8
(*c*)	1·6–2·7	2·27–3·51	0·90–1·46	1·8–3·1	1·76–3·09	0·17–0·47	1·0–1·5	2·2–6·6	0·9–2·2
(*d*)	1·6–2·6	2·40–3·59	0·90–1·37	2·2–3·3	1·84–2·74	0·13–0·43	0·9–1·5	2·7–6·5	1·0–2·2
(*e*)	1·6–2·5	1·76–2·36	0·77–1·07	1·8–2·7	1·63–2·40	0·13–0·39	0·7–1·2	3·5–7·4	0·9–2·0

grade (7), which has not been observed in Inland specimens, these three major habitat regions are represented by at least a few members of each grade. The percentages for the five habitat subdivisions of the Coastal samples do not reveal any tendency for the degree of hairiness to be influenced by the prevailing environmental conditions.

TABLE III. *Leaf pubescence (percentage plants occurring in each arbitrary grade)*

Grades ...	1	2	3	4	5	6	7	8	9	No. of plants
Inland (total)	1·3	21·4	14·0	8·1	24·1	24·3	0·0	4·2	2·6	1290
Island (total)	1·5	19·4	9·7	13·4	6·1	33·1	1·0	7·1	8·8	537
Coastal (total)	3·8	74·3	11·2	3·0	2·6	2·7	1·2	0·9	0·4	2203
Habitat (*a*)	5·0	82·0	5·5	1·0	2·5	1·7	0·2	1·5	0·5	596
(*b*)	4·6	72·5	10·7	4·4	1·5	2·2	0·7	2·2	1·1	542
(*c*)	2·4	77·1	14·6	1·9	2·7	0·8	0·2	0·3	0·0	625
(*d*)	1·8	68·9	14·5	4·7	3·6	4·1	0·3	0·9	1·2	338
(*e*)	6·9	38·2	15·7	6·9	17·6	4·9	1·0	6·9	2·0	102

Leaf spot. Spotting of the leaves due to the presence of anthocyanin pigment in some of the epidermal cells has been observed in 20% of the total number of plantains examined from habitats in Britain. The spots are usually small and of a deep wine-red colour, but their size, number per unit area and colour intensity vary greatly. The lack of spots is a recessive condition.

Spotted-leaved plants are much more frequent in some populations than in others, e.g. the percentage frequency of spotted plants in the Inland samples ranges from 0 to 45, in the Island from 6 to 53 and in the Coastal from 1 to 55. The average percentage occurrence for these three regions is, however, approximately the same being 17·9 for the Inland samples, 21·0 for the Island and 21·1 for the Coastal. As far as can be ascertained from the available data, the distribution of spotted plants is independent of latitudinal influence, percentages of 54 and 55 having been recorded from the North of Scotland and the South of England respectively. Nor can the conditions of the chosen habitat categories be said to control the distribution of spotted plants; for instances two apparently similar habitats have yielded samples of which one had 6% and the other 54%.

III. CHARACTER VARIABILITY

Habitat samples

For analytical purposes it is necessary to treat the individual characters as separate entities, although it is appreciated that the survival of a habitat population is dependent on the sum total of its

characters being at least tolerant of the prevailing environment. In comparing the variability of the different characters, different units of measurement have been used, e.g. mm., cm., inches, etc., therefore the variability has been expressed by the coefficient of variation (C). Table IV presents the average C values for each character for the major and minor habitat categories, and in addition the total average C value for each character. From an examination of the latter it will be seen that the variability is greatest for the leaves and scapes and least for the floral parts. That is, variability in relation to size is greatest for those characters which differentiate habitat populations. Moreover the C values for a given character tend to decrease as the size of the organs concerned increases, i.e. variability in relation to size is least in populations with large constituents. The reason for this may perhaps be more readily appreciated by reference to an example taken from the mean and range values for the character scape length (Tables I and II). In Table II the range for the (*a*) habitat category is given as 7–51 cm. and for the (*d*) category as 29–69 cm. The mean values for these two habitats are 28 and 46 cm. respectively, showing that for habitat (*a*) the mean of the samples was four times larger than the smallest variate, while for habitat (*d*) it was only one and a half times greater.

Although the coefficient of variation is a useful statistic for the comparison of the variability of a character in relation to size, yet it cannot be employed in assessing the actual variability of characters. When the size and habit of growth characters for the individual samples were examined it was found that their mean values were correlated with their C values, the coefficients of correlation being: scape length $-0{\cdot}80$, scape height $-0{\cdot}84$, spike length $-0{\cdot}64$, leaf length $-0{\cdot}82$, leaf breadth $-0{\cdot}76$, and the ratio scape spread : height $+0{\cdot}73$. These correlation values show that, as the means of the organs increase, and as the samples become more erect, the C values decrease. But for the characters spike length, leaf breadth and the ratio scape spread : height the mean values are also positively correlated with the standard deviations thus: spike length $+0{\cdot}66$, leaf breadth $+0{\cdot}75$, and scape spread : height $+0{\cdot}86$. Therefore to arrive at the actual variability it is necessary to discount the effect of absolute size.

In making the comparison between the variability of Inland and Coastal samples it has been possible to eliminate the effect of differences in absolute size owing to the Inland mean values falling within the Coastal range. The average C values have been deter-

TABLE IV. *Coefficients of variation (summarized)*

Habitats	HbG	ScS/ScH	ScS	ScH	ScL	ScTh	SpL	ScV	LfL	LfB	LfTh	LfS	LfH	LfS/LfH	SpD
Inland (total)	33·2	17·4	16·9	18·7	15·6	13·5	20·1	47·1	19·6	28·9	14·4	23·2	29·5	23·8	15·1
Island (total)	36·9	30·0	21·6	27·1	20·0	15·6	26·0	59·6	25·0	31·6	13·4	32·6	35·6	31·3	13·1
Coastal (total)	32·8	20·2	20·4	21·2	17·2	14·0	21·4	54·5	21·3	28·6	15·5	27·5	34·9	27·4	13·6
Habitat (*a*)	44·3	24·5	23·3	27·9	22·1	16·1	25·0	64·5	28·3	36·5	18·5	35·9	41·3	31·6	14·1
(*b*)	34·9	20·9	21·7	23·7	18·4	14·4	21·6	61·1	23·1	31·9	15·8	31·5	39·2	31·3	13·3
(*c*)	21·0	18·3	20·8	14·6	12·8	12·8	19·6	50·9	15·3	21·3	14·7	19·5	30·2	27·4	14·9
(*d*)	18·8	14·2	15·5	13·4	12·6	11·8	17·7	36·6	15·3	20·4	12·3	18·0	27·9	21·0	12·9
(*e*)	42·5	25·3	17·3	25·6	18·2	14·4	22·7	54·3	19·4	28·1	14·6	26·4	24·0	13·5	13·2
Average C value	33·6	21·0	19·6	21·4	17·2	14·1	21·8	53·2	21·4	29·2	14·9	27·1	33·4	27·0	13·9

Habitats	BrL	BrB	BrIx	SepL	SepB	SepIx	SdL	SdB	SdIx	AL	ATL	BrL/SepL	ScL/SpL	ScL/LfL
Inland	13·2	10·6	11·3	7·9	9·7	10·3	6·4	6·7	6·6	9·3	20·0	10·8	15·9	16·5
Island (total)	14·1	11·7	12·1	9·7	—	—	8·5	7·9	8·9	9·9	20·2	11·2	19·8	20·8
Coastal (total)	13·0	10·0	10·5	7·9	8·7	9·3	7·9	7·8	7·3	8·5	21·8	10·3	16·2	15·8
Habitat (*a*)	12·9	9·0	11·2	7·3	—	—	8·9	8·2	7·8	9·1	23·6	11·1	15·8	18·8
(*b*)	14·3	11·1	10·4	8·2	9·9	10·5	8·5	8·3	8·1	9·7	21·4	11·1	16·0	17·4
(*c*)	12·5	9·4	11·1	7·0	7·5	9·1	8·1	8·3	7·4	8·1	20·6	9·8	17·6	12·6
(*d*)	12·1	9·9	9·4	7·7	8·6	9·0	6·7	7·0	5·9	6·9	20·5	9·7	15·6	12·3
(*e*)	12·4	10·8	12·4	9·3	9·0	9·4	5·6	6·7	7·1	8·5	23·8	10·7	16·6	15·9
Average C value	13·2	10·4	10·9	8·2	9·1	9·7	7·6	7·5	7·3	8·9	21·2	10·6	16·7	16·8

mined for the Inland samples and for an equivalent number of Coastal samples with an average mean value closely approximating to that of the Inland samples. For example, the average of the mean values for scape length for the nine Inland samples is 34·82 cm. (Table I), and for the particular nine Coastal samples taken it is

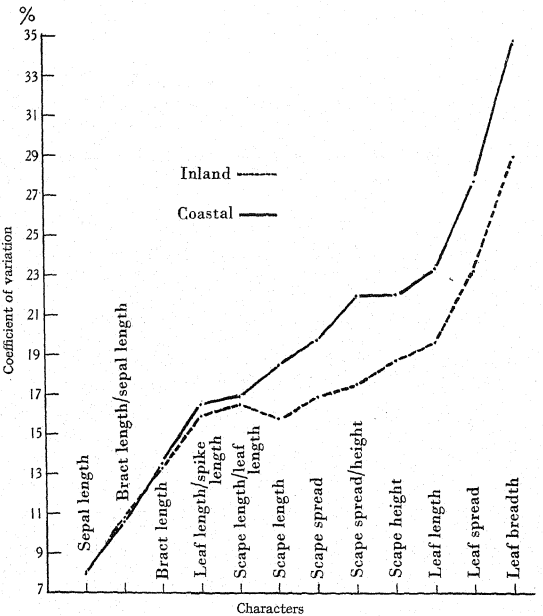

Fig. 2. The variability of Inland and Coastal populations compared.

34·99 cm.; the average C values for the Inland samples is 15·59% (Table IV) in comparison with 18·48% for the Coastal samples. In Fig. 2 the Inland and Coastal average C values, calculated in this manner, are given for the characters previously found to be influenced by habitat conditions, and also for the floral parts bract length and

sepal length. It is seen that when equivalent mean values are considered the Inland samples tend to have a lower variability than the Coastal.

When assessing the actual variability of the Coastal habitat categories, however, difficulty is experienced because equivalent size groups are not available.[1]

Wild growing populations

To obtain data with reference to the variability of populations when growing under natural conditions the habitats of samples PMN 32 (category (*a*)) and PMN 54 (category (*c*)) were again sampled. Individual plants were dug up and the leaf and scape characters were measured, the same procedure being observed as for the measurement of these characters under garden conditions. These samples are referred to as PMN 32 (2) and 54 (2). In Table V the coefficients of variation are given for the characters examined, together with the increase in size resulting from cultivation expressed as a percentage of the size of the equivalent wild sample. It will be seen from the latter that the increase under cultivation is greater in the case of PMN 32. Moreover, in comparison with PMN 32, PMN 32 (2) shows a significant decrease in variability relative to size in the characters scape length, leaf length and leaf breadth, a reduction which is not found in the case of PMN 54 (2).

TABLE V

	% increase in mean size under cultivation		Coefficient of variation			
			Habitat (*a*)		Habitat (*c*)	
Character	PMN 32 and 32 (2)	PMN 54 and 54 (2)	PMN 32	PMN 32 (2)	PMN 54	PMN 54 (2)
Scape length	403	68	24·6 ± 1·52	18·3* ± 1·33	10·3 ± 0·75	9·9 ± 0·69
Spike length	549	44	27·7 ± 2·09	23·7 ± 1·75	16·9 ± 1·25	18·7 ± 1·36
Leaf length	600	26	34·4 ± 2·38	25·2* ± 1·89	12·8 ± 0·93	11·5 ± 0·82
Leaf breadth	152	11	44·6 ± 3·70	20·7* ± 1·51	20·1 ± 1·49	21·1 ± 1·54
Average	426	37	32·8	22·0	15·0	15·3

* $D/E_d > 3$.

The evidence suggests that the modificatory influence of the wild environment can effect a considerable reduction of variability in relation to size and that this is most pronounced when dwarf growth is favoured by the environment.

[1] Since going to press B. Day and R. A. Fisher have published details of a method for the comparison of variability in populations having unequal means (*Annals of Eugenics*, 7, (4), 333–348, 1937).

IV. THE EFFECT OF AN ARTIFICIALLY IMPOSED ENVIRONMENT

The data furnished in Table I established the fact that racial differentiation had taken place in response to the prevailing environmental conditions in respect of the size and growth-habit characters. However, they do not supply evidence relative to the stage of development at which the plants were most sensitive to the selective influence of the environment. In order that the behaviour of parts of the same population growing under different conditions might be analysed experimentally a plantain sample, a mixture of three habitat collections, was sown in combination with herbage species in three series of eight plots as illustrated in Table VI. The series A and C were sown with, and B without, a nurse crop of oats.

TABLE VI. *Arrangement of plots and rate of seeding in lb. per acre*

Pl. maritima seeding rates	Series of plots A	B	C	Other species seeding rates
3	I	I	I	*Phleum pratense* 25
5	II	II	II	*Trifolium repens* 2
10	III	III	III	
10	IV	IV	IV	*Ph. pratense* 25
10	V	V	V	*T. repens* 10
10	VI	VI	VI	*Cynosurus cristatus* 20
10	VII	VII	VII	*C. cristatus* 10, *Festuca ovina* 10
10	VIII	VIII	VIII	*F. ovina* 20

During the following seasons (1929–1933) series A and B were closely cut each week, while series C was allowed to grow undisturbed. Throughout the course of the experiment natural seeding of plantains was prevented by the periodic removal of ripening scapes.

Establishment and subsequent elimination of plantains

Year 1929. In April the number of plantains occurring in each of the eight plots of the three series was determined. Before recording the plant counts a border of 20 sq. ft. round each plot was discarded, leaving an area of 4×4 ft. which was then divided into sixteen equal parts. The numbers per square foot were recorded separately. The plot counts expressed as percentages of the number of *viable* seeds sown are given in Table VII, cols. 2–4. It was found that no statistically significant differences occurred between equivalent plots for the two series A and C sown with a nurse crop. The data for these two series have therefore been combined in Table VIII, col. 2. This

table shows that when the combined series AC is compared with the no-nurse-crop series B the only significant difference (differences exceeding three times their errors) occurring between equivalent plots are those between the thinly sown I plots and between the *Trifolium repens* plots (V). The absence of a cover crop in the B series allowed the clover to grow much more vigorously. Similarly within a series

TABLE VII. *Establishment and subsequent elimination in the series A, B and C*

	Plants established % 1929			Plants eliminated (%) between the years								
				1929–31		1931–34		1929–34				
Plots	A	B	C	A	B	A	B	A	B	C		
I	7	3	10	78	76	100	100	100	100	100		
II	10	14	11	64	76	98	83	96	96	100		
III	14	16	14	86	86	44	70	92	96	100		
IV	20	22	23	5	0	3	28	8	28	80		
V	9	3	10	74	98	74	100	93	100	100		
VI	28	22	26	23	24	7	19	28	38	78		
VII	32	27	31	25	14	0	0	25	14	56		
VIII	33	28	30	14	7	0	0	14	7	38		

TABLE VIII. *Mean number of plantains per sq. ft.*

	Year 1929			Year 1931			D/E_d 1929–31	
Plots	AC	B	D/E_d	A	B	D/E_d	AC and A	B and B
I	2·7 ± 0·26	1·1 ± 0·20	4·9	0·5 ± 0·15	0·0 ± —	—	6·6	—
II	5·5 ± 0·46	7·7 ± 0·77	2·5	1·8 ± 0·28	1·9 ± 0·36	0·2	6·9	6·8
III	12·3 ± 0·63	13·3 ± 1·24	0·7	1·7 ± 0·29	1·9 ± 0·28	0·5	15·3	9·0
IV	18·3 ± 1·77	18·4 ± 1·54	0·4	16·2 ± 1·04	19·3 ± 1·07	2·1	1·0	0·5
V	7·8 ± 0·66	2·9 ± 0·70	5·1	1·9 ± 0·32	0·1 ± —	—	8·0	—
VI	23·3 ± 1·20	18·6 ± 1·58	2·4	18·7 ± 0·79	14·1 ± 0·90	3·8	3·2	2·5
VII	26·9 ± 1·45	23·1 ± 1·94	1·6	20·8 ± 1·08	20·0 ± 0·80	0·6	3·4	1·5
VIII	26·5 ± 1·75	24·0 ± 1·54	1·1	23·9 ± 0·77	22·4 ± 0·82	1·3	1·4	0·9

the percentage establishment of sea plantains has been appreciably affected by the relative abundance of *T. repens*. For example, the clover plots III and V of the AC series contained significantly fewer plantains than the remaining plots which received the same quantity of plantain seed. Moreover plot V with the greatest seeding of clover possessed the lowest number of plantains. The figures for plots IV, VI, VII and VIII do not differ significantly from each other. In the B series plot V alone is distinctive, plot III having this time a count statistically similar to plots IV and VI. As in the AC series plots IV, VI, VII and VIII do not attain a significant difference.

Year 1931. This year the plantains in the C series were not counted, as accurate figures could not be obtained owing to the height of the herbage. The two mown series A and B, however, were

again examined and it was found that during this 2-year period elimination of plantains had taken place. From Table VIII it can be seen that the reduction in numbers is parallel in both series and also, from the last two columns of the same table, that in the majority of plots the reduction is statistically significant. Plots III and V have suffered a significantly greater elimination than plots IV, VI, VII and VIII. Since 1929 the clover in plots III had developed rapidly and the difference in number of plantains between these plots and plots V is no longer significant. In Table VII the relative rate of plantain elimination for the various plots since 1929 is given, the number of plantains eliminated being expressed as percentages of the number established in April 1929.

Year 1934. In April 1934 it was no longer possible to determine the number of plantains in the plots by a surface examination owing to the density of the herbage. The counts were, therefore, obtained by digging the plots and separating the plantains from the other vegetation. An examination of the plants showed that the effect of the continual cutting of the AB series had been greatly to reduce the above ground parts, and also to reduce the root length by approximately 30% and the root diameter by 20% in comparison with the unmown plots. As counts per square foot were no longer practicable, the differences in numbers between the series A, B and C and between their component plots cannot, in this instance, be assessed with statistical accuracy. Nevertheless the differences between the counts recorded per plot in 1934 and the numbers per plot surviving in the years 1931 and 1929 provide a guide to the course of elimination, and they have been expressed in percentage form in Table VII. During the last 3-year period the rate of elimination in series A and B had not been retarded appreciably as far as the clover plots were concerned. On the other hand elimination had either ceased or had been maintained at a low level in the plots IV, VI, VII and VIII.

Besides the data for the mown series AB, the 1934 records provide information relative to the uncut series C. From Table VII it will be seen that in the clover plots the elimination of plantains is complete in the unmown series C and almost complete in the mown. In the grass plots, however, the rate of elimination in the C series greatly exceeds that in the AB series.

These results may be summarized briefly as follows: (1) establishment in no case exceeded 33% of the viable seeds sown, (2) elimination was not limited to the seedling stage, (3) rate of elimination was influenced by environment.

To determine whether this elimination of plantains had been selective or merely at random, samples from equivalent plots were compared. Only plots VII and VIII contained a sufficient number of plants for a test, and the former were chosen. Eighty plants were taken at random from plots VII C and VII B respectively and each group was seeded in isolation. The remaining plants from both plots were kept until the spring of 1935 when single cuttings of equal size were taken off 102 plants from each plot. These cuttings together with 102 seedlings raised from each isolation, making a total of 408 plants, were transplanted into the garden for examination in 1936. A sample of the original seed sown on the plots in 1928, which was to have acted as a control, failed to germinate.

As size and growth habit differences were the characters which, in the wild, distinguished the grazed populations from the ungrazed, these characters were chosen in making a comparison of the material from the plots.

TABLE IX

	Plants from			Progeny of plants from		
Character	Plot VII (B)	Plot VII (C)	D/E_d	Plot VII (B)	Plot VII (C)	D/E_d
LfH	$4{\cdot}22 \pm 0{\cdot}126$	$4{\cdot}52 \pm 0{\cdot}130$	1·69	$3{\cdot}96 \pm 0{\cdot}099$	$4{\cdot}59 \pm 0{\cdot}130$	3·91
LfL	$25{\cdot}07 \pm 0{\cdot}456$	$25{\cdot}25 \pm 0{\cdot}380$	0·30	$24{\cdot}33 \pm 0{\cdot}416$	$25{\cdot}21 \pm 0{\cdot}515$	1·33
ScH	$14{\cdot}60 \pm 0{\cdot}238$	$14{\cdot}96 \pm 0{\cdot}246$	1·05	$14{\cdot}23 \pm 0{\cdot}268$	$14{\cdot}92 \pm 0{\cdot}249$	1·88
ScL	$39{\cdot}86 \pm 0{\cdot}536$	$40{\cdot}32 \pm 0{\cdot}513$	0·62	$39{\cdot}74 \pm 0{\cdot}560$	$40{\cdot}82 \pm 0{\cdot}542$	1·39
SpL	$9{\cdot}72 \pm 0{\cdot}177$	$10{\cdot}13 \pm 0{\cdot}188$	1·59	$9{\cdot}75 \pm 0{\cdot}170$	$9{\cdot}99 \pm 0{\cdot}144$	1·06
ScS/H	$1{\cdot}25 \pm 0{\cdot}027$	$1{\cdot}22 \pm 0{\cdot}025$	0·83	$1{\cdot}32 \pm 0{\cdot}029$	$1{\cdot}28 \pm 0{\cdot}021$	1·25

The results of the garden tests which are presented in Table IX show that in all the characters studied there is a slight though insignificant tendency for the plants of series C origin to be larger and more erect than those of series B origin. Furthermore, the progeny tests provide corroborative evidence of this tendency. Considering that the elimination which occurred in the unmown plot was four times as great as that in the mown, this negligible effect of the environment is somewhat surprising. It would appear from these data, therefore, that in spite of a differential death-rate the elimination of growth-forms has been predominantly at random, After 6 years it might have been expected that the differences between the two series would have been greater if the vegetative phase of the individual plants had been subject to the environmental selection imposed upon it. A conclusion that may reasonably be drawn is that since in the material employed the vegetative phase was not particularly sensitive to the imposed conditions, population differentiation in "wild" grazed and ungrazed habitats is mainly the result of

differences in the amount of seed produced by the different growth-forms, e.g. decumbent scapes would suffer less than erect ones from the attacks of grazing animals. The plan of the present experiment did not allow of this assumption being put to the test.

Breeding experiments have shown that, in both the growth-habit and size characters, populations respond rapidly to rigorous artificial selection. Unfortunately the plantain material employed in these experiments was accidentally destroyed before the records could be completed. However, the results of a parallel series of experiments with two habitat samples of diploid *Phleum pratense* are available. The components of these two samples, spaced at 2 ft. intervals, were examined during their second season, and two pairs of similar phenotypes, representing extreme variates in respect of growth-habit, i.e. decumbents and erects, were selected from each sample. These pairs were crossed *inter se* under control and their progenies were in turn examined in their second summer. From these, pairs of decumbent plants were selected from the decumbent progenies and erects from erects, and these also were crossed *inter se*. This procedure was repeated and the results are presented in Table X. It will be seen that the mean values of the first generations differ very appreciably from those of the habitat samples.

When two phenotypes, one from each of the extreme progenies derived from the second habitat samples, were crossed, the resulting population possessed values in respect of panicle height and the ratio panicle height : spread approximately midway between the parent populations, the respective values being $23{\cdot}25 \pm 0{\cdot}112$ and $1{\cdot}54 \pm 0{\cdot}011$. The cross decumbent × erect and the reciprocal yielded similar results.

TABLE X. *Rate of differentiation following rigorous phenotypic selection within two habitat samples*

		Decumbent selections		Habitat samples	Erect selections	
Character	Sample	Third gen.	First gen.		First gen.	Third gen.
Mean values:						
PH	1	$10{\cdot}8 \pm 0{\cdot}25$	$11{\cdot}7 \pm 0{\cdot}27$	$14{\cdot}0 \pm 0{\cdot}18$	$22{\cdot}6 \pm 0{\cdot}17$	$22{\cdot}3 \pm 0{\cdot}22$
	2	—	$11{\cdot}7 \pm 0{\cdot}22$	$19{\cdot}4 \pm 0{\cdot}28$	$28{\cdot}1 \pm 0{\cdot}27$	$28{\cdot}7 \pm 0{\cdot}15$
PH/S	1	$0{\cdot}3 \pm 0{\cdot}01$	$0{\cdot}7 \pm 0{\cdot}02$	$1{\cdot}1 \pm 0{\cdot}03$	$1{\cdot}9 \pm 0{\cdot}01$	$1{\cdot}9 \pm 0{\cdot}03$
	2	—	$0{\cdot}4 \pm 0{\cdot}01$	$1{\cdot}3 \pm 0{\cdot}03$	$2{\cdot}1 \pm 0{\cdot}03$	$2{\cdot}8 \pm 0{\cdot}02$
Coefficient of variation:						
PH	1	$18{\cdot}8 \pm 1{\cdot}69$	$24{\cdot}9 \pm 1{\cdot}68$	$16{\cdot}6 \pm 0{\cdot}94$	$7{\cdot}4 \pm 0{\cdot}52$	$11{\cdot}7 \pm 0{\cdot}71$
	2	—	$18{\cdot}9 \pm 1{\cdot}41$	$16{\cdot}8 \pm 1{\cdot}05$	$9{\cdot}7 \pm 0{\cdot}69$	$7{\cdot}5 \pm 0{\cdot}39$
PH/S	1	$25{\cdot}2 \pm 2{\cdot}33$	$30{\cdot}0 \pm 2{\cdot}08$	$40{\cdot}0 \pm 2{\cdot}58$	$13{\cdot}0 \pm 0{\cdot}93$	$15{\cdot}4 \pm 0{\cdot}94$
	2	—	$19{\cdot}1 \pm 1{\cdot}43$	$29{\cdot}2 \pm 1{\cdot}91$	$13{\cdot}4 \pm 0{\cdot}97$	$10{\cdot}1 \pm 0{\cdot}53$

PH = panicle height in inches.
PH/S = panicle height/spread.

V. Discussion

Initial differentiation of populations

The differences between plantain populations have been shown to be almost exclusively dependent on the frequency with which certain expressions of quantitative characters belonging to continuously graded series are represented. This lack of discontinuity makes it necessary to assess differences in terms of character mean values, and it is helpful before discussing problems of classification to consider some of the agencies responsible for altering these values.

From an examination of habitat populations in the wild, it would appear that the variety of hereditary forms constituting a population is to some extent masked by the reaction of the population as a whole to external environmental stimuli. While such fluctuating variability often exaggerates the precision of adaptation, it must actually permit the reproduction of variates whose potential values deviate from the reproductive optimum of the population. At the same time, ability on the part of variates to fluctuate in response to habitat conditions must add considerably to the stability of populations by preventing temporary changes of environment from affecting permanently their hereditary constitution. It has been demonstrated, particularly in the case of the continuously distributed coastal populations, that persistent environmental differences influence the distribution of certain hereditary characters, e.g. growth-habit and size. But it is difficult to determine to what extent ecological barriers of this kind interfere with the *dispersal* of the genetic factors responsible for the appearance of these and other characters. From the fact that the expression of certain characters such as leaf colour is often similar in adjoining habitats and that pollen from one habitat must sometimes invade another, it may be inferred that the isolation afforded by virtue of habitat differences alone is not absolute. Such incomplete isolation, while tending to decrease the degree of differentiation between populations for some non-adaptational characters, may actually increase the divergence in respect of others. For example, where physical or biotic environmental selection is in the direction of either tallness or dwarfness, the occasional acquisition from other habitats of genes inducing greater height or more extreme decumbency would facilitate a still greater differentiation between populations, under the influence of Natural Selection.

Nevertheless, in spite of the opportunities afforded the non-adaptational characters to spread under strictly ecologically main-

tained isolation, many coastal populations exhibit a marked individuality in respect of characters which have no apparent ecological significance. In coastal regions, habitats are continually being destroyed and new ones created, and the colonization of the latter introduces the possibility that some degree of spatial isolation during the process affects the distribution of non-adaptational characters. The colonization of recently formed habitats can, however, be best observed in inland districts. The trend of inland migration, as pointed out previously, is primarily from mountain habitats into small areas on the borders of streams and paths, i.e. into recently formed and more or less isolated and unoccupied habitats. Bearing in mind the heterogeneous nature of the average population, it is highly improbable that populations resulting from the sporadic invasions of habitats by a few seeds from a single locus would represent the genetic constitution of the parent population, or even be themselves of similar constitution. So long as random elimination of variates remained at zero, the introduced "neutral" genes would in all probability increase numerically at their original frequencies, provided that they were equally free of interference from those responsible for differential reproductive rates. But as a habitat reached its maximum complement random elimination would become increasingly severe and, judging by the rarity of seedlings in fully occupied habitats, seed from variates of low frequency would stand a poor chance of establishment. Thus migration followed by random elimination may play its part in establishing in a comparatively short space of time distinctions of no adaptational consequence.

Similar hereditary expressions of growth-habit and of size may be found dominating dissimilar habitats under certain circumstances, although, as has been shown, the distributions of these characters are susceptible to environmental control. For example, sample PMN 16 was collected from a habitat lying at an elevation of 100 ft. at the base of Ben Cruachan. This habitat supported a population composed of plants phenotypically almost twice the size of those occupying the upper slopes of the mountain. But when samples from both situations were grown under similar conditions in the experimental garden the size difference was no longer evident, a circumstance which suggests the absence of certain size factors in this locality.

The availability of characters is doubtless a potent force in determining the extent to which ecological differentiation is realized in isolated regions. For example, decumbent growth-habit might

conceivably dominate a sheltered ungrazed situation if by chance it was the only growth-type available, although normally its ecological importance is greatest under conditions of extreme exposure and heavy grazing. If, as appears certain, the majority of the present lowland populations represent isolated parts of long established, presumably previously selected mountain populations, it is not surprising that the differentiation of ecologically sensitive characters is less noticeable in the inland habitats than in the coastal regions where habitat contacts increase the chances of populations acquiring genes from elsewhere. By way of illustration *Saxifraga* (*Bergenia*) *crassifolia* L., which "grows copiously" at low levels in the Altai mountains and "is found as scattered individuals in sheltered places even up to the tree limit", maintains its *lowland* characteristics at the high altitudes (Turesson, 1931, p. 338). On the other hand, an alpine type of *Solidago virgaurea* L. "is found in *regio alpina* of the East-Altaian mountains, differing in regard to height and earliness from the lowland type of the same region, while in the Alps and Carpathians no such alpine type has been found" (p. 345). Turesson concludes that this "is no doubt due to the absence in these regions of the biotypes necessary for the differentiation of that type" (p. 345). Such distributional anomalies indicate the need for care in assigning an adaptive value to any character.

Classification of local differentiation

It has never been seriously suggested that all the discernible differences between individual plants should be named. However, the naming of every discernibly differentiated sea plantain population would be as unjustifiable as the naming of differences between their component plants, because few populations are identical in composition. If as Cockayne & Allen (1927, p. 259) say "the goal of taxonomy is the power to discover the status of the individual plant", the units of taxonomy would require to be determined at a level more distinctly demarcated than that at which the initial differentiation recorded in this paper takes place. Considering the nature of the differentiation of the plantain populations in Britain it would indeed be difficult to assign with any degree of accuracy an individual specimen to its appropriate population, e.g. individual specimens possessing common characteristics may occur in populations which are dissimilar by virtue of their constituents being represented in different proportions. Of the thirty-two samples examined from habitats in Britain, all except three possessed decumbent growth

forms, distinguished by having the scapes peripheral and decumbent, only five contained truly erect types, and all contained forms intermediate between these two extremes. When, however, the local distribution of growth-habit was examined it was found that the local population differences were not necessarily associated with the presence of peculiar growth-forms but were due to changes in the numerical relationships between types. Moreover, the examination of the hereditary composition of population samples from certain recognizably different habitats demonstrated that in respect of the continuously graded character series, growth-habit and size, this local differentiation is effected in response to local environmental conditions. Plants with peripheral and decumbent scapes can be readily distinguished from those which have scapes erect and general. If these forms had occurred in each local habitat in unvarying proportions, it might have been sufficient only to record their existence. In reality, however, it is necessary to consider not only the proportional distribution of these extreme forms of the character series, but also the effects of ecological conditions upon the distribution of the series as a whole. When it is found that the characters which differentiate one population from another belong to a continuous unbroken series, any attempt to describe the population differences verbally in terms of such characters naturally tends towards an arbitrary description of the more or less recognizable steps within the series. But since all the components of a series are presumably liable to be acted upon by Natural Selection, an artificial grouping within the series might conceivably be detrimental to the proper interpretation of the forces operative in Nature. The accuracy of recording the distribution of the variates belonging to a continuous series can be greatly increased by recording their presence in numerical terms either by the direct measurement of the characters of the individual variates, e.g. as in assessing the character scape spread : height ratio, or by devising a numerical scale for the recording of observable but ill-definable differences, e.g. the evaluation of habit grade.

In addition to the differentiation of populations accounted for by the uneven representation of ecologically controlled characters, qualitative differences are also to be found. Such qualitative differences as the presence or absence of spots and hairs on the leaves fall within this category, but in reality they merely represent definable steps in quantitative series, e.g. the difference between glabrous leaves and those exhibiting a few hairs being very slight indeed. Similarly the absence of the leaf spot character represents the end-point of a

series culminating at the other extreme in the coalescence of numerous deeply pigmented spots. The distribution of spotted-leaved plants is apparently independent of ecological selection although the frequency of spotted plants varies much locally. The data previously given show that some habitat samples may entirely lack spotted plants, e.g. PMN 55 from a spatially isolated population; this, and not the fact that plants can be separated into spotted and non-spotted categories, is an important taxonomic observation from the evolutionary standpoint.

Here then is the initial population differentiation resulting from the operation of different causes; in the case of the growth-habit and size characters it is in response to environmental conditions, and in that of leaf spot apparently it is in consequence of chance spatial isolation of non-spotted plants. Since the criterion for delimiting habitat populations rests mainly on the differential numerical representations of characters, no clear-cut morphological distinction exists between habitat populations. However, on the basis of the Natural Selection theory the degree of morphological distinctness should be greatest as the ecological differences influencing the distribution of the characters become more pronounced. On the other hand, the character differentiation resulting from the chance isolation of a part of a larger population may also vary considerably in degree and may perhaps be greater, even from its inception, than the differences between many ecologically isolated populations. For example, the lack of spotted-leaved plants in a habitat is more striking than is a difference of, say, a few centimetres in scape length. In an intrafertile assemblage, any character difference which indicates the process by which populations in the wild maintain their identity can be justifiably regarded as being of at least as great value in an experimental taxonomic system as the degree of morphological distinctness. In other words, when dealing with such dynamic units as local populations the process of differentiation is of more importance taxonomically than the degree of discernible morphological differences exhibited at any given time. The units of experimental taxonomy, if they are to represent the natural subdivisions of a wild population, will therefore require to be based on the causes which initiate differentiation rather than on the extent of the observable differences between populations. That is, the character differences will be diagnostic of populations belonging to the same taxonomic unit but will not be the determinants of taxonomic status.

If names are to be given for reference purposes to different populations of the same taxonomic status the question arises whether (1) distinctive habitat populations should each receive a name indicative of the habitat, or (2) the names of different populations should have reference to the proportional representation of one or more ecological indicator characters, e.g. growth-habit. In the first case the greater the habitat diversification the greater would be the need for names, e.g. the five coastal groups of habitat populations studied, and probably many more, would be eligible; in the second the number would be constant because all the differences between the habitat populations could be recorded in terms of the three most easily recognizable growth-habit types. Although plant size also follows an ecological sequence, growth-habit is to be preferred as a basis for the nomenclature because it can be identified with considerable accuracy without recourse to actual measurement.

Using Turesson's (1922) terminology, the ecologically differentiated races of *Plantago maritima* within Britain can be classified according to the predominating type of growth-habit into three ecotypes, *decumbens*, *ascendens* and *erecta*. The diagnostic characters are represented diagramatically in Fig. 3, which also depicts the relationship between the largest and smallest form of each growth-type.

The relationship between the named ecotypes and the populations occupying the Inland habitats and the habitat categories (*a*) to (*e*) is given in Fig. 4. The position of the populations on the habit grade scale and the size relationships of their component plants are based on the mean values for the characters habit grade and scape volume given in Table I.

There still remains the population (PMN 55) which lacks the leaf-spot character. As the spotted condition is almost universally distributed in Britain regardless of ecological differences, its lack is presumed to be due to the chance introduction of recessive plants into this spatially isolated habitat. Since population differentiation of this kind is not ecotypic, units separated in this manner should not be confused with ecotypes. It is therefore proposed to give this population the taxonomic status of a geo-ecotype (Gregor 1931) and apply the name *immaculata*.

Ecotypes of the same species situated in different regions are, however, likely to exhibit differences due to an uneven geographical distribution of ecologically tolerant characters. Even in a region the size of Britain there is evidence that on the average the Inland

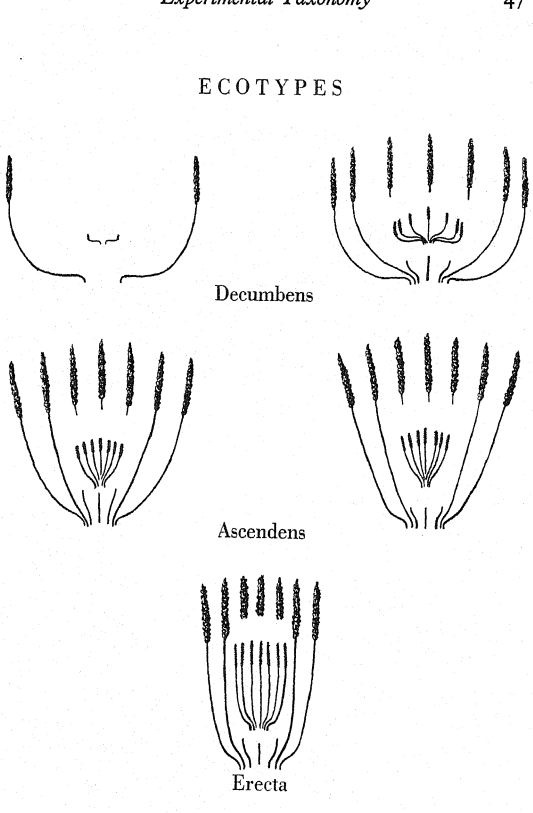

Fig. 3. Ecotypes of *Plantago maritima*.

ecotypes have sepals which are narrower in proportion to their length than those of the Coastal populations (Table I). Moreover the Coastal populations of the Mainland are less hairy than the ones from the Hebridean Islands (Table III). Geographical restriction of this kind affects the characters of all the ecotypes within the area concerned, and in a system of classification it is important that the existence of these geographical phases or regional concentrations of characters, whether or not they merge insensibly one with another, should be mentioned, perhaps in a preface, without necessarily giving them the status of a taxonomic unit.

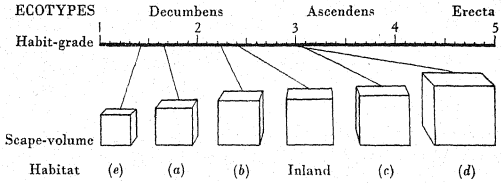

Fig. 4. The relationship between the ecotypes and their habitats.

VI. SUMMARY

1. *Plantago maritima* occurs more or less continuously round the coast of Britain. Inland its distribution is sporadic, radiating mainly from spatially isolated mountain loci.

2. Population samples from Inland, Island and Coastal habitats have been examined in the experimental garden at Corstorphine.

3. Population differentiation of a hereditary nature is shown to take place in response to the prevailing environmental conditions. The differences between populations are largely dependent on the frequency with which certain quantitative characters belonging to continuously graded series are represented.

4. Differentiation is most marked for the growth-habit and size characters, such as the habit and size of the scapes and the leaf dimensions. The characteristics of the floral parts may, however, be influenced to some extent by general plant size, and thus indirectly by habitat conditions.

5. It is suggested that in classifying races emphasis should be transferred from the degree of morphological distinctness exhibited by populations to the processes which initiate differentiation, and

that taxonomic *status* should be accorded the units which are maintained by particular natural circumstances irrespective of the extent to which they differ morphologically.

6. Three ecotypes, *decumbens*, *ascendens* and *erecta*, and one geo-ecotype, *immaculata*, have been named.

I am greatly indebted to my colleague Mr J. M. S. Lang for helping me to collect the data from which the foregoing conclusions have been drawn, and to Prof. J. R. Matthews of Aberdeen, for supplying information relative to the distribution of *Ph. maritima* in the north-east of Scotland.

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THE AUTECOLOGY OF *ZOSTERA MARINA* IN RELATION TO ITS WASTING DISEASE

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(With Plates I and II and 2 figures in the text)

THE Zosteraceae as defined by Hutchinson (1934) is a family entirely confined to the sea and including only two genera, *Zostera* with a wide distribution in the temperate parts of both hemispheres, and *Phyllospadix* which, according to Ostenfeld (1927), is confined to western North America and Japan. These two genera may be readily distinguished when in flower, as the former has linear spadices and monoecious flowers, and the latter broad lanceolate spadices and dioecious flowers. When not flowering the more caespitose rhizome, clothed in fibrous remains of leaf sheaths, serves to distinguish *Phyllospadix* from *Zostera*.

In European seas there are three species of *Zostera*, all of which occur in Britain, and three other marine genera of flowering plants, *Posidonia*, *Cymodocea*, and *Halophila*, are also represented, each by a single species in the Mediterranean. *Posidonia oceanica* has a tufted habit, and great masses of stiff fibres derived from leaf sheaths clothe the rhizome; *Cymodocea nodosa* resembles *Zostera marina* to some extent, but may be distinguished by the leaves being serrulate towards the tip, the rhizomes reddish instead of brownish, and the roots arising singly instead of in clusters. It is probable that some of the more southern localities given for *Z. marina* in the Mediterranean should apply to *Cymodocea*. The remaining species, *Halophila stipulacea*, occurs only at Rhodes in the Mediterranean region, and was probably introduced there, being carried by a ship passing through the Suez Canal (Fritsch, 1895). It may be readily distinguished from the other marine angiosperms found in European seas by its stipitate leaves.

The Zosteraceae are unique among marine angiosperms in their strictly temperate distribution, the marine representatives of other families being essentially tropical or subtropical in their occurrence, only rarely extending far enough north or south for a slight overlap with the Zosteraceae to occur.

The species of *Zostera* can be quite clearly distinguished by a combination of morphological and anatomical characters which are described at length by Setchell (1933) and Sauvageau (1891 *a*, *b*), and which it will be convenient to summarize here in the form of a brief description of the genus and its European species.

Zostera L. *Spec. Pl.* ed. 1 (1753), 968

Perennial, submerged, marine herbs with a horizontal, monopodial rhizome rooting at the nodes and bearing alternate distichous linear ligulate leaves with sheaths closed or open. *Squamulae intravaginales* occur on the stem just above the insertion of each leaf (Arber, 1920). Sterile lateral shoots, often soon separating from the primary shoot by the decay of the old rhizome. Flowering shoots annual, erect, sympodial, flattened, simple or branched, internodes long. Flowers monoecious, borne on one side of a spadix formed of the flattened end of the axis and enclosed in a spathe formed of the always split sheath of a more or less reduced leaf. Male and female flowers alternating in two rows (Eichler, 1875). Male flowers reduced to a single stamen (Pl. I), female flowers a single one-seeded ovary with a single style and two stigmas (Pl. I). Pollen thread-like. *Retinaculae*, or small bract-like organs, are present in some species at the base of the stamens, and are sometimes considered to represent a very reduced perianth.

The genus was divided into two sections by Ascherson (1860) and a third section was subsequently added by Setchell (1933). The European species of section *Alega* have branched flowering shoots, longitudinally costate seeds, *retinaculae* absent or only rarely occurring at the bottom of the spadix, closed leaf sheaths and bundles of strengthening tissue in the outermost layers of the cortex. The one European representative of section *Zosterella* has unbranched flowering shoots, each bearing a solitary spadix, smooth seeds, *retinaculae* always present, leaf sheaths open, and strengthening tissue only in the inner layers of the cortex.

The European species may be distinguished by means of the following key:

(1) Seeds ribbed, leaf sheaths entire, flowering stems branched...(Section *Alega*) (2)
Seeds smooth, leaf sheaths split, flowering stems unbranched...*Z. nana* Roth (1827) (Pl. II) (Section *Zosterella*)

(2) Leaves usually 0·5–1·0 cm. broad, stigmas as long as style, seed 3·5 mm. long...*Z. marina* L. (1753)
Leaves usually 0·2 cm. broad, stigmas half as long as style, seed 2·5 mm. long...*Z. Hornemanniana* Tutin (1936)

The leaves of *Zostera marina* have for a long time been an article of commerce and have recently increased in importance since their value as a sound and heat insulating material has become known (Griffiths, 1922; F.I.B. 32). In Canada, and to a lesser extent in Europe also, the plant is of value as food for wild game birds, as an important factor in the formation of a suitable environment for various fishes, and as a natural binder that prevents shifting of sand and mud banks in harbours. Between 1930 and 1933 *Z. marina* died out nearly completely in most places on the Atlantic coasts of North America and Europe. The disease which caused this widespread destruction will be discussed later, but it will be seen that in addition to the effect on the plant itself, and on the numerous associated animals and epiphytic algae, the disease has had considerable economic effects. In Canada alone, according to a typescript report by Harrison F. Lewis,[1] the annual financial loss at a conservative estimate has been 152,100 dollars.

AUTECOLOGY OF *ZOSTERA MARINA* L.

(*a*) *The habitat*

Z. marina is tolerant of a fairly wide variety of bottoms ranging from soft mud to gravel mixed with coarse sand. The most usual type of bottom on which it grows in the British Isles is firm muddy sand which is often covered with a layer of coarse sand. It seems probable that it obtains most of its supply of water and mineral salts by absorption over the leaf surface, and that the roots serve chiefly for anchorage. This is suggested by the ease with which water is lost to the air from the leaves and by the rather poor development of the root system. The type of bottom on which the plant occurs in a particular habitat is closely correlated with the exposure of that place to wave action. For *Zostera* to be able to grow in any particular locality the bottom must consist of sufficiently coarse particles to be stable under the action of the waves which normally occur in that place. On the south coast of England, for example, *Zostera* occurs in patches on the Mewstone Reef to the east of Plymouth, where it is often exposed to fairly heavy seas, and here the bottom consists mostly of gravel. In the mouth of the River Yealm not far away, where the bar breaks the main force of the seas, the bottom is firm

[1] This, and a number of other manuscript reports and letters have been deposited at the Marine Biological Association's Laboratory at Plymouth.

sandy mud, while in Prinstead Creek, Sussex, which is almost land-locked, the plant thrives on mud which is so soft that a man sinks up to the knees in it. On the east coast of England in the region of the Wash, there are extensive banks of sand and mud in shallow water on which *Zostera* is only prevented from growing, as far as can be seen, by the continual shifting of the bottom.

The amount of light the plant receives is correlated with the degree of wave action and type of bottom, and consequently the depth down to which it can grow depends partly on these factors. It is very difficult to get an accurate idea of the illumination on various *Zostera* beds throughout the year as it depends on so many factors. During a storm turbidity is high in all types of habitat, as the muddy bottom in sheltered beds is stirred up by the small waves occurring in such places just as the gravel and sand bottom is stirred up by the larger seas in the more exposed places. When the storm is over however, a ground swell persists for days in the exposed places and keeps the turbidity high while in sheltered places settling occurs much more rapidly. It is not possible to give exact figures for the amount of light cut off by this turbidity, but Kitching *et al.* (1934) state that after a storm in summer in a locality with a sandy bottom visibility under water was only about 2 ft., and in a Scottish loch with a mud bottom under similar circumstances work in a diving helmet in shallow water was impossible owing to the lack of visibility. The general effect was described as resembling a London fog. It is evident from this that the degree of exposure to wave action has a very great effect on the illumination, and that even a slight swell, such as commonly occurs in summer, will considerably reduce the light in exposed localities. The deposition of silt which adheres to the diatoms and other small algae and hydroids epiphytic on all but the youngest leaves of the plant also cuts off the light in places where the water is often turbid. In still, calm water light is fairly rapidly absorbed and this limits the depth to which the plant can grow even under the most favourable circumstances. Round the coasts of the British Isles it does not appear to occur more than 4 m. below L.W.O.S.T. (low water ordinary spring tides), while in the clearer water and more intense insolation of the Mediterranean it occurs at depths down to 10 m. (Fiori e Paoletti, 1896–8). The height above L.W.O.S.T. to which it can grow is limited by the type of bottom, by the local characteristics of the tidal cycle, and by the resistance of the plant to drying. The latter point will be discussed later in this paper. The type of bottom has a large effect, as the surface layers of sand dry rapidly while mud stays

wet much longer when exposed to air, and holds numerous pools of water usually only a few millimetres deep, but sufficient to prevent the plant from drying up during the period of low tide. Consequently, on a muddy bottom *Zostera* beds may be found at levels where they are exposed at nearly all tides, while usually on a sandy bottom they are only exposed for a short time at L.W.O.S.T. or are always submerged, except perhaps at equinoctial spring tides, though in such cases the limit may be imposed by other minor factors. Among these factors may be mentioned wave action, which is much more severe when the plant is exposed to a breaking wave on the shore, than when it is under a few feet of water. Local tidal conditions also affect the upper limit of *Zostera* growth. In the neighbourhood of the Isle of Wight where the tidal cycle has two maxima separated by a very high minimum and followed by a low minimum a plant growing, for example, at half-tide mark is exposed for a much shorter time than would normally be the case. The time of day at which L.W.S.T. occurs is approximately constant for a given locality so that this also has an effect on the upper limit of *Zostera*. When it occurs in the middle of the day the drying is much greater for an equal length of exposure than when it occurs in the morning and evening. Consequently *Zostera* grows at higher levels on the west coast of Scotland where L.W.S.T. occurs at about 6 a.m. and 6 p.m. than it does in south Devon where it occurs at about noon and midnight.

The temperature of the sea has no large and rapid fluctuations, comparable with those on land, and so the temperature relations of a marine plant can be studied with much greater ease and accuracy than those of a terrestrial plant. The temperature range is rather greater in estuaries, semi-land-locked bays and in large areas of very shallow water, than in what may, for convenience, be regarded as normal inshore waters. In the latter in south England a minimum temperature of 7–8° C. occurs in February and March. From that time till August or September there is a fairly steady rise, more rapid in May and June than earlier and liable to checks or even slight falls if the weather is very bad. 10° C. is usually reached at the end of April or early in May and 15° C. at the end of June or early in July, with a maximum of 16 or rarely 17° C. in August, or occasionally as late as September. The deviation from the figures given here is less than 1° C. in most years. These figures give a good idea of temperature conditions on the *Zostera* beds in Cawsand Bay, and on the north side of Drake's Island near Plymouth, which are two of the areas parti-

cularly studied during this investigation. The other two *Zostera* beds on which frequent observations were made are in the Yealm estuary and the Salcombe estuary also in south Devon, and here temperature conditions show local peculiarities. These estuaries are both inlets of the sea into the landward end of which only a small volume of fresh water runs, so that the salinity is not appreciably reduced and their character is essentially marine. In both cases the seaward end is protected by a bar which stretches nearly across the narrow rocky mouth and breaks the force of the sea. Higher up there are extensive mud-flats which get heated up at low tide on a sunny day and warm the water as it flows over them to such an extent that in summer it is usually 2 or 3° C. hotter than the open sea. In winter the temperature is about the same as in the open sea, but in summer maxima of 20° C. may be reached, and temperatures of 16 or 17° C. may occur even as late as October when the weather is sunny. In the places where the *Zostera* beds are situated in these localities these high temperatures are attained only on the ebb, as the plant grows near the seaward end of the estuaries and gets unheated water from the open sea on the flood.

Temperatures of a similar order obtain throughout most of the European range of the plant. In the North Sea the heating in summer is a little greater, owing to the shallowness of large areas of water, and it tends to be cooler in winter, particularly in the more northern parts. On the Portuguese coast the temperature is usually rather higher since it is farther south and gets more intense insolation and also a greater mixture of the warm water which flows out of the Mediterranean. Onshore winds at times bring in cold Atlantic water and very much reduce the temperature. In the Mediterranean itself the temperature range is notably different. The February temperature in the Straits of Gibraltar is well above 10° C. and in August over 20° C. Observations made by ss. *Xauen* (1932) in lat. 35 N. long. 5 W., just inside the Straits of Gibraltar, will serve as an example of the annual temperature range in this region. In February the surface temperature was 15·48° C., in May 17·11° C., in September 22·60° C. and in November 18·50° C.

On the Atlantic coast of North America temperature conditions differ even more widely from those along the majority of European coasts. The range is far greater and consequently the rise in spring and fall in autumn are much more rapid. Observations taken off the coast of North Carolina by the M.Y. *Atlantis* in 1932 in lat. 36 N. long. 75 W. give the surface temperature in April as 11·6° C. and in

September as 26·80° C. (C.P.I., 1932). Farther north, near New York, the minimum may be as low as 3° C. and the maximum 26° C. or a little higher. The temperatures on *Zostera* beds in shallow or very sheltered water would be even more extreme.

The relatively small variations which occur in the chemical composition of the sea do not, as far as can be determined, have any appreciable effect on large plants such as *Zostera*, though they are of great importance to the small planktonic algae. These will therefore be neglected, and the only other habitat factor which will be considered is salinity. This is almost constant in the sea as a whole, varying usually between about 34 and 36 g. of dissolved salts per litre. Detailed figures of this, and also of temperatures, for the Atlantic and the North Sea may be found in the annual *Bull. Hydrograph.* published by the Conseil Permanent Internationale pour l'Exploration de la Mer. In estuaries and in the Baltic the salinity may fall as low as 26 g. per l. in places where *Zostera* grows, while in the strip of water nearly enclosed by Chesil Beach, Dorset, another habitat of *Zostera*, it may rise owing to summer evaporation, to 42 g. per l. *Z. marina* was kept in the laboratory for a considerable period in salinities ranging from 10 to 40 g. per l. without apparent harm, and withstood immersion in fresh water for 2 days.

(*b*) *The plant*

Z. marina grows in pure societies of varying extent, from small patches a foot or two across to great beds the size of which can be measured in acres. The size of the bed may depend, of course, on its age, but it is also limited by obvious factors such as depth and the nature of the substratum. There is good evidence that a well-established *Zostera* bed can extend from a stable substratum over an unstable one, which is too easily disturbed by waves to allow direct colonization by seedlings or fragments of rhizome. When this occurs the dense matted rhizomes stabilize the sand, and on all *Zostera* beds there is a tendency for sand to accumulate and the bed to become raised above the surrounding bottom. The long leaves of the plant break the force of the currents and small waves, and check their scouring action. The actual raising of the level does not seem to be a serious cause of death to the plant, and does not as a rule exceed a few inches. The rhizome branches freely, but no great length of it is alive at any given time. The growth of the rhizome and many other points in the life history of the plant have been described in detail by Setchell (1929) for var. *latifolia* Morong, which is the common Pacific

form. I am indebted to this paper for much accurate information which has been checked, in most details, for the typical European form. The leaf is said to reach a length of 1 m. (Graebner, 1907), though half this is more usual, and to vary in breadth between 2 and 5 mm. or up to 12 mm. in var. *latifolia*. The length and breadth seem to vary more or less proportionally, the very narrow leaves being only a few centimetres long. Very small leaves seem to be an indication of unsuitability of the habitat or of disease, though the leaves on flowering stems are always narrower and shorter than those on sterile shoots. The long summer leaves die in the autumn, leaving the shorter winter leaves which grow slowly or not at all till the following spring, when they in turn die and are replaced by the longer summer leaves. The number of leaves produced annually per plant and an estimate of the total annual yield in Danish waters is given by Petersen (1913).

Setchell (1929) has shown that the yearly cycle of growth and reproduction in *Z. marina* var. *latifolia* is controlled by the changes in temperature during the year, and his results have been found to hold with only minor exceptions for var. *typica* in European waters. Active vegetative growth begins when the sea temperature reaches 10° C., generally speaking at the end of April or beginning of May in south England, and continues with increasing rapidity as the temperature rises to 15° C. Flowering does not begin till the temperature reaches 15° C., and when it exceeds 20° C. the flowers and immature fruits die and the flowering stems eventually become detached from the plant. The start of active vegetative growth is rather difficult to observe exactly, but as far as can be made out a temperature of 10° C. is very nearly if not quite the minimum necessary. The commencement of flowering is an easier matter to observe and it coincides very closely with the attainment of 15° C., which usually occurs in July in south England. That 15° C. is the limiting temperature for flowering is indicated by observations made near Plymouth in 1935 and 1936. In June 1936 the temperature on the *Zostera* bed was just over 14° C. and at the same time in 1935 about 1° higher. The lower temperature in 1936 delayed the start of flowering by about a month. When the temperature continues above 20° C. for a time the vegetative as well as reproductive activities of the plant cease, and when the temperature falls in the autumn Setchell has found that no renewal of growth occurs between 20 and 10° C., but that the plant is dormant until the temperature rises again to 10° C. in the spring. He suggests that this may be due to the persistence of what he terms heat rigor till

it changes over into cold rigor when the temperature falls below 10° C. His alternative suggestion is that the fall in temperature in the autumn is too rapid to allow visible growth to occur. The fact that *Zostera* grows in the Mediterranean in places where the temperature rises well above 20° C. and never falls below 10° C. indicates that heat rigor can give place to active growth without intermediate cold rigor, and suggests that it is either a matter of time, or that the decrease in light intensity during the fall in temperature prevents the renewal of growth in the autumn, but that this occurs in the spring with increasing illumination irrespective of whether the temperature has fallen in the interval below 10° C.

Very similar relations hold for *Z. Hornemanniana* and *Z. nana*, but no detailed work has been done on these species. The flowering season is considerably longer, lasting usually from June to November, while at the most it extends only from July to September for *Z. marina* The difference in habitat is responsible for this as the two former species are chiefly estuarine and grow at relatively high levels, usually on mud. The vigorous and healthy plants, particularly those of *Z. Hornemanniana*, live as a rule in shallow depressions in the mud, and the water held by these at low tide gets warmed to temperatures well above 15° C. in the summer whenever there is sunshine, even though a cold wind is blowing. The period of exposure in 24 hours is very long owing to the high level at which the plants grow, so that for a considerable part of every sunny day the temperature of the water surrounding the plants is suitable for flowering. *Z. Hornemanniana*, like *Z. marina*, tends to accumulate mud round its rhizomes and, though the mound produced is not more than 3 cm. high at most, this is sufficient to bring the plant out of water at low tide, and check flowering and growth to such an extent that the plant ultimately dies. Erosion follows the death of the plant and conditions may again become suitable for recolonization. Thus this community is another example of a community in which a succession does not follow from the action of the plant on its habitat, but there is merely a cyclic change in the vegetation.

Experiments of a simple type were made to compare the resistance to drying of the three species, and it was found that the leaves of *Z. marina* withstood exposure to air and sunlight for only about half the time that those of the other two species did. This difference was very much increased when the water was drained off basins of *Z. marina* growing in sand and *Z. Hornemanniana* growing in fine silt. Neither species was noticeably affected by rain during periods of

exposure. Several attempts were made to transplant *Z. marina* to beds of *Z. Hornemanniana*, but in each case the transplants died rapidly. The shoots of *Z. marina* are fairly rigid at the base and when exposed at low tide stick up into the air for a centimetre or two, while those of *Z. Hornemanniana* are much less rigid and lie flat, so that in spite of the shallow pools of water on the mud, the former are killed by the prolonged drying while the latter remain moist and survive. Even a short period of drying kills the flowers of any of the species, the stigmas being particularly susceptible.

Pieces of living rooted rhizome of *Z. marina* a few centimetres long with a tuft of leaves at the apex are often found washed up in the neighbourhood of *Zostera* beds after storms, and such fragments are usually capable of growth in suitable conditions and are apparently an important means of distribution for the plant. A comparison of the effectiveness of seeds and detached shoots as means of distribution was made in the case of *Z. Hornemanniana*, which is very similar to *Z. marina* in this respect. Records of the occurrence of *Z. marina* at some places on the east coast of England are based on washed-up shoots, but as there is no record of the plant actually growing in the locality these had probably been carried by sea for some distance (Butcher, 1934).

In the winter of 1935–6 samples of mud were collected from the neighbourhood of *Z. Hornemanniana* beds and the number of seeds in them counted. Approximately the top 2 cm. of mud from areas each 0·25 sq. m. in extent was removed with a shovel and passed through a sieve of 1 mm. mesh to wash out the mud, and the number of seeds in the residue counted. By taking these samples at different times throughout the winter the viability of the seeds, as well as their dispersal, was measured. *Z. Hornemanniana* was used for this purpose as it was impossible to collect an adequate number of samples from *Z. marina* beds owing to the low level at which they grow. The samples were found to be fairly uniform so only four areas of 0·25 sq. m. each were collected on each occasion in November, December, January, February and April. In November and February additional samples were taken starting at a distance of 50 cm. from the plant. The results are given in Table I. These results indicate that the mortality among the seeds themselves, exclusive of the young plants, is very high, and that the dispersal of the seeds is by no means great, even after the rough winter weather. This was confirmed by counting seedlings in March, April and May, when an average of 2 seedlings per sq. m. was found in the 50 cm. strip round the beds, and only

TABLE I. *Number of seeds per sq. m. Trevol, River Tamar, 1935–6*

Month	Total	Living	Germinated	Dead†
	0–50 cm. from plants			
November*	67	25	2	40
December	88	18	3	67
January	55	3	2	50
February	71	7	1	63
April	42	0	1	41
	50–100 cm. from plants			
November	10	1	0	9
February	13	0	0	13

* Plants in the neighbourhood still flowering.
† Seeds were considered to be dead when only the testa remained.

occasional ones beyond this. The greatest distance from a mature plant at which a seedling was found was 1·32 m. The viable seeds definitely tend to collect in the small hollows in the mud, while the dead seeds, which rapidly become reduced to empty testas, are more uniformly scattered. Each bed of the plant is usually surrounded by a slight depression and this accounts in part for the high concentration of living seeds close to the plants.

As already pointed out, the flowering season of *Z. Hornemanniana* is much longer than that of *Z. marina* even under the most favourable conditions found in northern European waters, so the seed production of *Z. marina* is lower and the number of surviving seedlings is very small, even in favourable years, and in many years none. This conclusion is supported by field observations, and by the scarcity of definite records of seedlings of *Z. marina* in the literature.

These field observations were checked by experiments which also helped to give a correlation between the germination of the seeds of the two species, and indications of the influence of temperature and light on the germination.

The summer of 1935 was favourable for the flowering and fruiting of *Z. marina*, and in August and September a supply of seeds was collected by keeping fruiting stems of the plant, obtained chiefly by dredging and diving, in tanks of circulating sea water in the light and picking out the ripe seeds each day. The seeds on each spadix ripened in no definite order, the ripening extending often over a period of a week or more. The seeds are contained in the green membranous ovary wall and when they are ripe this wall splits and the seed is extruded. The dehiscence of the fruit occurs chiefly on sunny days, and fruits kept in the dark do not dehisce, fail to germinate and

eventually rot. The mechanism of dehiscence seems to be that the ovary wall becomes weakened and is finally split by the slight pressure of the gas produced inside it during photosynthesis, as a bubble, presumably of oxygen, could always be seen escaping when dehiscence occurred. The seeds of *Z. marina* and *Z. Hornemanniana* are cylindrical with rounded ends, generally light brown in colour, though sometimes white or blue-grey, with numerous longitudinal ribs. Those of the former are 3·5 mm. long, and of the latter 2·5 mm. long.

The seeds, in lots of 50 chosen at random, were placed on filter paper in glass finger-bowls of filtered sea water and covered with a glass plate. The bowls were kept under various combinations of conditions, the water being changed frequently and the number of seeds which died or germinated counted at intervals. The results are summarized in Table II. The number of seeds obtainable for these

TABLE II. *Numbers of germinants*

Conditions ...	Daylight 9–13° C.		Daylight 16° C.		Dark 12–15° C.		Dark 14–16° C.
Species ...	*M**	*H*†	*M*	*H*	*M*	*H*	*M*
November	0	0	0	1	0	0	1
January	0	0	2	2	3	1	1
February	6	0	0	1	1	4	7
March	4	10	0	0	1	8	5
April	0	9	0	0	2	7	0
Totals	10	19	2	4	7	20	14
% germinated	20	38	4	8	14	40	28

* *M* = *Z. marina*. † *H* = *Z. Hornemanniana*.

experiments was too small to allow definite conclusions to be drawn from the results, but it is evident that the percentage germination in the two species under similar conditions is similar and very low, but probably always somewhat lower in *Z. marina* than in *Z. Hornemanniana*. The low germination is not due to the failure of the embryo to develop during ripening, as in 20 ripe seeds of *Z. marina* that were dissected the embryos appeared quite normal.

The following conclusions are also suggested by the figures available, but cannot be put forward with any degree of certainty without additional experiments on larger numbers of seeds: A constant high temperature causes earlier germination but a much lower percentage of seeds germinate. The seeds of both species appear to germinate about equally well in the light and in the dark. *Z. Hornemanniana* germinates about a month later than *Z. marina* under

similar conditions. The experiments were continued until the beginning of July, but there was no further germination after April. A few non-germinants rotted between November and February, and in March and April most of the remainder rotted. From the results of counting seeds in the field it seems that a high proportion rot very rapidly under natural conditions, or else the remains survive for one or more years in a recognizable state. The later rotting in the laboratory is probably accounted for by the fact that only well-ripened and apparently healthy seeds were used for the experiments, and the filtering of the sea water removes considerable numbers of bacteria and fungal spores. An attempt was made to find out if the seeds are eaten to any appreciable extent by the numerous waders which visit the *Zostera* beds at low tide, but this was defeated by weather and the wariness of the birds. In the Herbarium of the New York Botanical Garden there is a packet containing over 1000 seeds of *Z. marina* taken from the oesophagus of a bird shot in Maine in 1906, but there does not appear to be any similar evidence from this side of the Atlantic.

The seeds which rotted in the laboratory were always full of bacteria, and in one case a very coarse fungal mycelium was found, but no fructifications were produced. Other groups of 50 seeds were subjected to various treatments to try to stimulate germination. In one case the testa was chipped round the plumule. This caused a rapid elongation, but it was of very limited duration and the seedlings all died in a few weeks. Two other groups of seeds were churned up with sand and water, one for 24 hours and the other for 7 days, but no significant difference in germination was obtained. It was found that exposing the seeds of *Z. Hornemanniana* to the air at room temperature on a dry substratum for 30 min. caused no damage, but that an exposure of 60 min. or more killed the plumules of all the seeds. Seeds kept at 0° C. in sea water for 36 hours germinated normally, but others kept under these conditions for 21 days died. The ease with which the seed is killed by drying makes it very unlikely that it can ever be carried for very long distances in a viable condition on the feet of sea birds.

The first sign of germination is the splitting of the testa in the neighbourhood of the plumule, which then elongates and produces the first leaf which is of the same type as the leaves of the mature plant and has several other young leaves enclosed within its sheath. Two very small adventitious roots appear from the first node at this stage, but do not elongate till later (Text-figs. 1 and 2). The primary root, though present in the embryo, never develops and the young

plant is anchored firmly by the numerous unicellular hairs which grow out from the superficial cells of the cotyledon and often reach a

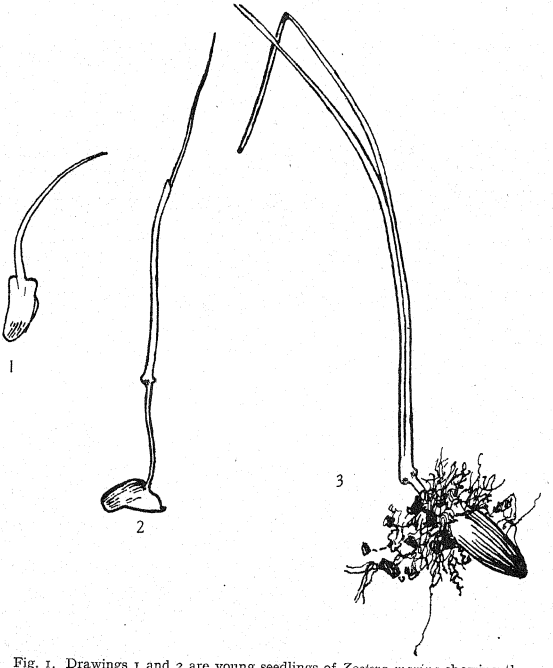

Fig. 1. Drawings 1 and 2 are young seedlings of *Zostera marina* showing the origin of the adventitious roots, the long epicotyl, and the first leaf. No. 3 is a seedling of *Z. Hornemanniana* showing the origin of the adventitious roots and the long unicellular hairs produced from the superficial cells of the cotyledon.

length of about 1 cm. Other leaves appear and by the time two or three have been developed the adventitious roots elongate, penetrate the soil and develop root-hairs. The epicotyl persists for some time

after this, but slowly withers and becomes detached after a few months. In the early stages of germination it was found that in the case of *Z. marina* the seedlings are injured or even killed by rapid changes of temperature of only a few degrees, in this respect resembling the young of many marine animals which are usually very susceptible to slight, rapid changes of temperature. In the early stages also, the seedlings may get buried or their shoots bruised or broken in rough weather. Apart from these comparatively unimportant accidents there seem to be no causes of mortality on a

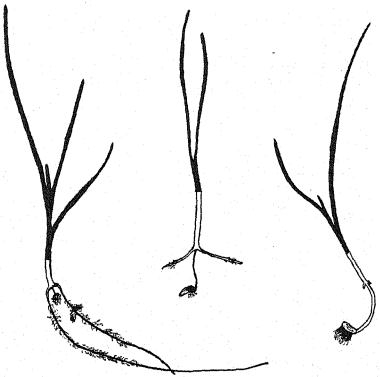

Fig. 2. Seedlings of *Zostera Hornemanniana* showing development of adventitious roots. About natural size.

large scale in nature at this stage, otherwise it is very unlikely that regeneration from seed would ever occur.

Pieces of rhizome of *Z. Hornemanniana* were often found at distances of 20 m. or more from any large patches of the plant; these are capable of establishing themselves and forming fresh colonies, so there is no doubt that these detached pieces are far more efficient from the point of view of distributing the plant and colonizing fresh areas than the seeds. It is often difficult at first sight to distinguish between young plants growing from fragments of rhizome and seedlings, the presence of a length of old rhizome in the one case, or the remains of the seed or epicotyl in the other, being essential for a

sure distinction. A good series of drawings illustrating the seedling stages and later development of *Z. marina* var. *latifolia* Morong is given in Setchell's paper (1929, Figs. 2–32).

(c) *The disease*

About 1931 attention was called to a disease of *Z. marina* which was causing extensive mortality of the plant on the Atlantic coast of the United States, and from that time onwards there have been numerous typescript reports and published notes about the disease in that region (Huntsman, 1932; Cottam, 1933, 1935; Lewis & Taylor, 1933; Mounce, 1933; Stevens, 1933; Taylor, 1933; Renn, 1934, 1935, 1936 *a*, *b*). Since the spread and symptoms of the disease have been described in detail in these and other papers, a brief summary will be sufficient here. In 1930 there was some evidence of a local decline in the abundance of the plant, which was followed by a marked diminution in 1931. A year later it was reported as having disappeared practically completely from Beaufort, North Carolina, in the south to Nova Scotia in the north, with the exception of a few estuaries and harbours where there were still good stands. In 1933 the scarcity spread northward still farther and included practically all the localities in Canada where the plant was known to occur. In Europe the disease was first noticed in France (Fischer-Piette *et al.* 1932) and in the British Isles (Cotton, 1933) at Plymouth, where the plant is believed to have begun to decrease towards the end of 1931, at Abbotsbury, Dorset and at Castletownshead, Co. Cork. The disease became general in 1933 in the British Isles and was also reported from Sweden (Lönnberg & Gustafson, 1933; Blegvad, 1933), Denmark (Blegvad, 1934; Petersen, 1933), Holland (Spierenburg, 1933), Germany (Wohlenberg, 1935) and Portugal.

Although it was estimated that in many countries 90% of the plant had been destroyed in 1933 and 1934 there is at present no sign that the remaining 10% is disappearing, and in the British Isles at least there is a slight, though quite definite improvement.

The symptoms of the disease as I have seen it in many localities round the British Isles are exactly the same as those described from several different countries. Small brown spots develop on the leaves, and these soon spread and become darker, covering large portions of the leaves, which eventually become detached from the plants. No dredge that I have tried digs sufficiently well to uproot more than occasional pieces of the plant, and as the diseased leaves become detached much more readily than the healthy ones it is impossible to

get an accurate idea of the condition of a *Zostera* bed by this means. The rhizomes as well as the leaves show a discoloration, though not as early or as distinctly, and they frequently survive for a year or more after the disease becomes evident. A vivid description of the effect of the disease in the course of a single year is given by Wohlenberg (1935). Since this appears to be the only description of a *Zostera* bed before and after the onset of the disease, and my own observations did not begin till 1934, it seems desirable to quote it here:

"Es konnten damals (Sommer, 1932) in Sylter Gebiet noch keine Anzeichen einer Erkrankung festgestellt werden. Vielmehr war die Entwicklung der Seegraswiesen vor dem Königshafen noch so üppig, dass ich in diesem Gebiete bei Niedrigwasser die grösste Mühe hatte, mit dem Ruderboot durch die dicht bei dicht im Wasser flutenden gefunden, langer Blätter voranzukommen....Im Sommer des folgenden Jahres (1933) waren die Bestände jedoch bereits erheblich gelichtet. Die Seegraswiese hatte im Juli zwar noch ein frischgrünes Aussehen, aber die Bedeckung des Bodens war nicht mehr vollständig, was sie sonst in diesem Gebiet zu sein pflegte. Die Blätter erreichten nur etwa $\frac{1}{4}$ der üblichen Länge und waren ausserdem bedeutend schmaler....Die letzte Untersuchung im September (1934) v. Is. ergab, dass die Seegraswiesen vor dem Königshafen zerstört und nicht mehr als Formation zu bezeichen sind."

The remarkably wide occurrence of the disease and the fact that according to the records it appeared in Europe one, or possibly two years, after it was first noticed in America, gave rise to the suggestion that the pathogenic organism had travelled across the Atlantic. It was pointed out by Cottam (1934) that a similar, though perhaps less severe, epidemic had occurred on several previous occasions among the *Zostera* beds of the United States and probably also in Europe, at different times in the two continents. This fact rather suggests that the occurrence of the present epidemic in Europe and America simultaneously is due to a coincidence rather than to the spread of the disease from one continent to the other.

Workers in many countries have made investigations into the disease with a view to finding the pathogenic organism responsible for it. In France, Fischer-Piette *et al.* (1932) reported the discovery of a bacterium in the diseased tissues, but no definite proof of its pathogenicity has been given. In the United States C. E. Renn, working at Woods Hole, found that a species of *Labyrinthula* was always present in the diseased parts of *Zostera marina* from both American

and European sources, and the writer was shown this organism, which is somewhat difficult to detect, by Dr F. K. Sparrow, Jr. in diseased *Zostera* at Plymouth (Renn, 1934, 1935, 1936 *a*, *b*). Renn has shown quite definitely that the *Labyrinthula* can invade the living tissues of the plant and cause the spotting of the leaves characteristic of the disease. A small patch of *Zostera Hornemanniana* in the River Tamar was found showing the dark spots on the leaves and the *Labyrinthula* was found associated with them, but the disease has since disappeared after killing out the infected patch, and it shows no sign of spreading in this species.

In Canada in 1934 Mounce & Diehl (1934) described a new species of *Ophiobolus*, *O. halimus* from *Zostera* and this has since been found in diseased *Zostera* from many parts of the British Isles, and, according to Renn, sparingly in the United States. By following the method described by Mounce & Diehl (1934) perithecia of this fungus were obtained by the present writer from every specimen of diseased *Z. marina* examined. A considerable number of specimens of *Z. Hornemanniana* and *Z. nana* were also examined by the same method for the presence of *Ophiobolus halimus* but it was uniformly absent in these species. A species of *Fusarium* was also found in one specimen but it is probably only of minor importance. The *Ophiobolus* was determined by Dr E. J. Butler and compared with authentic Canadian material. Petersen (1934) has found an *Ophiobolus* which appears to be identical with this species in Denmark, and has shown that it is a parasite capable of invading healthy tissue, and not a saprophyte as has been suggested. *O. maritimus* was erroneously reported to occur on *Zostera marina* but this was due to a misreading of the label of the type specimen (Tutin, 1934).

The suggestion has been made (Duncan, 1933) that the discharge of oil waste from ships was perhaps a cause of the destruction of *Zostera*, but this appears unlikely as the quantity of oil discharged has decreased recently, and also if this were the case, it would be expected that the beds in deeper water would be unharmed, while in fact they have suffered most.

Discussion

There are at least two parasites of *Zostera marina* which produce similar symptoms and which, as far as can be seen, are equally responsible for the disease. There is no good evidence that the infection has spread rapidly from one region to cover the large area at present affected, nor is there any suggestion that the parasites have

become suddenly much more virulent. The latter explanation might have served if only one parasite had been found, but it is hardly likely that two widely different organisms should have shown this change in behaviour at about the same time.

We are therefore forced to look for an explanation of the widespread disappearance of *Z. marina* in the ecology of the plant itself. It has been shown that the propagation of the plant is practically entirely vegetative, seed production being dependent on temperature conditions which are seldom realized over most of the area occupied by it. In the vegetative state growth is generally limited by the light intensity. If, as seems probable from their wide distribution, the parasites had been living in the *Zostera* for a considerable time but had done little harm while the plant maintained its full vigour, a slight check to the growth of the plant might be sufficient to allow the parasites to get the upper hand and cause widespread damage. Such a check could be most easily caused by a deficiency of sunshine, since, as has already been pointed out, light intensity is the limiting factor for vigorous growth in the majority of habitats. In the British Isles the year 1931–2 showed a sunshine deficiency of about 20% below normal, and no other year in the past ten showed a deficiency approaching this. The scanty figures available for other countries suggest that this unusual lack of sunshine was a general phenomenon, though in some countries it was less pronounced but of longer duration. As the plant seems to be incapable of recovering from the disease when once severely attacked, the sunny years following the onset had little effect except when the sea was warm enough for seed production. The seed has always been found to be free from both the *Ophiobolus* and the *Labyrinthula* so that seedlings have a chance of getting a good start before they become infected, and so it would be expected that regeneration would occur in places particularly favourable for the production and germination of seeds. At Salcombe, which has already been shown to be such a locality, seedlings have been found and the condition of the *Zostera* beds shows some improvement, though the original beds have continued to die out.

In the Mediterranean, where the temperature is suitable for seed production every year, and the light is brighter, no sign of disease has been reported, as would be expected. In the United States the temperature range is much greater than in Europe and consequently conditions suitable for regeneration from seed occur less frequently, so it is to be expected that the disease would be more severe and regeneration slower there, which is in fact the case.

The recovery of *Z. Hornemanniana* from an attack by the *Labyrinthula* may be due to its greater resistance to the parasite, but the complete killing out of the affected patch and the survival of the rest suggests rather that the more frequent reproduction of the plant from seed prevents the parasite becoming so generally distributed in this species as it has done in *Zostera marina*. It seems therefore that the evidence available justifies the putting forward of the ecological theory which fits with the various aspects of the disease that have come to my notice.

It is to be expected that the plant will recover from the recent epidemic rather slowly but more rapidly in the south than the north. The more exposed *Zostera* beds, and those in deeper water are to be expected to suffer more severely than those in sheltered places, and to depend for their regeneration chiefly on fragments of rhizome washed there from those in more sheltered places.

Summary

1. A brief account of the taxonomy of *Zostera* and other associated marine angiosperms is given.

2. The habitat factors of importance in the life of *Z. marina* are discussed.

3. The autecology of *Z. marina* and, to a less extent, of *Z. Hornemanniana* is described.

4. A brief account is given of the occurrence and symptoms of the epidemic disease of *Z. marina*.

5. The evidence for the disease being due to a bacterium, a fungus, a *Labyrinthula* or the discharge of waste oil from shipping is summarized.

6. It is suggested that the enfeeblement of the plant due to lack of sunshine in 1931–2 is the fundamental cause of the epidemic, and that recovery depends on the regeneration of the plant from seed and is therefore likely to proceed slowly.

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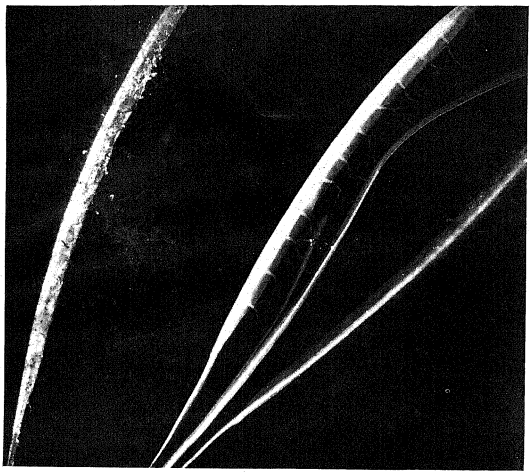

TUTIN—*ZOSTERA MARINA*

TUTIN—*ZOSTERA NANA*

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EXPLANATION OF PLATES I AND II

PLATE I

The upper photograph shows the female flowers of *Zostera marina* L., the lower, the male flowers with pollen being shed from the dehisced anthers.

PLATE II

Zostera nana in flower. Note the inflorescence in the centre of the photograph, and the two commonly associated organisms *Hydrobia* (left) and *Chaetomorpha* (bottom right). × 1·7.

PHYLOGENY AND POLYPLOIDY IN *ROSA*

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Taxonomic revision

The taxonomy of the genus *Rosa* has recently been clarified and the number of forms of specific rank has been considerably reduced. Many of the species are remarkably polymorphic and possess geographic races and ecotypes which are not yet understood. The recognition of well-defined series of parallel variations among the polymorphic species has elucidated the scope of intraspecific variation, potential and actual.

We are indebted to Dr G. A. Boulenger, who, after his retirement as one of the foremost zoological systematists of Europe, devoted his experience and taxonomic acumen to this difficult genus. Dr Boulenger has worked assiduously for fifteen years, revising first the European species (Boulenger, 1924–32) and then the Asiatic roses (1933–6). He distinguishes ten species in Europe and north-west Africa, and ninety-three species in Asia of which fifteen are found in other continents also. In my revision of the North American roses I recognized twenty native species, of these two also occur in Asia. This gives a total of 121 known species for the world (Boulenger, 1936 *a*), an enormous reduction over the number recognized fifty years ago.

When the chromosome number of a species is known Boulenger includes it in his description. He has divided the genus into seven sections. Although his classification was based purely upon morphological characters all polyploid species (except *R. gallica*) are found in the Section Eglanteriae and the majority of them in the Group Cinnamomeae-Caninae. This section also contains over 50% of the total number of species in the genus. It is most northerly in distribution and contains the most generalized and least evolved types.

Theory of descent from extinct arctic decaploid

The simplest and most primitive species in the Eglanteriae are polyploid and also arctic or alpine in range. The least specialized wild rose is *R. acicularis* Lindl., which is a circumpolar species containing

both hexaploid and octoploid strains. The hexaploid form extends farther south than the octoploid, but the northern limits of the two have not been defined. *R. pimpinellifolia* L. (the Scotch Briar) is the most generalized species of the mountains of Eurasia, and is the only native rose in Ireland: it is a tetraploid. These two species are the first to come into flower in the spring. Nearly all of the common species of America and Europe belong to the Eglanteriae. Many of them are polymorphic; they are successful species with extensive ranges and are in a state of rapid evolution and for these reasons they have received an excessive share of the attention of rhodologists. Just as the peculiar unbalanced polyploidy of the Caninae, common roses of Europe, led cytologists to consider the genus as a whole to be unique in behaviour, so the occurrence of primitive polyploids with high boreal distribution has influenced rhodologists to hypothecate an evolution of species in *Rosa* from generalized polyploid forms to specialized diploid ones: an hypothesis to which Boulenger (1936 *a*) has given his assent.

Täckholm (1922) postulated an extinct arctic decaploid rose to account for the unbalanced hexaploid *R. Jundzillii* Bess. which has seven pairs and twenty-eight univalent chromosomes at diakinesis, and Boulenger also thinks that this is necessary (1936 *a*). I have already pointed out (Erlanson, 1931) that this hypothesis is redundant since an unbalanced hexaploid with only seven paired chromosomes would result from the crossing of two hexaploids with only one set of homologous chromosomes in common. An octoploid crossed with a tetraploid could also give the same type of unbalanced hexaploid.

In 1925 Hurst put forward what Boulenger designates as "sa bizarre classification" based on the hypothesis of five differential septets of chromosomes in *Rosa* which were represented in nature by "five fundamental diploid species". It is evident that the number five was adopted because the author already accepted the hypothesis of an extinct ancestral decaploid. The geographic distribution of polyploid and diploid species led Hurst to conclude that modern rose species are all descended from this arctic decaploid.

This hypothesis is contrary to the established cytological principle that polyploidy is a secondary and derived condition. Commenting favourably on Hurst's theory of descent from higher polyploids Cockerell (1926) suggested that the hexaploid and octoploid forms had probably been built up from diploids by chromosome reduplication.

The following facts and considerations show that the acceptance

of a polyploid ancestry for the modern rose species is not the only alternative.

EVIDENCES FOR A POLYPHYLETIC DESCENT OF ROSE SPECIES

(1) *Primitive diploid relatives*

The primitive form *R. minutifolia* Engelm. is diploid with $2x = 14$. It has been placed in the genus *Hesperhodos* by Cockerell, a classification which is sustained by Boulenger and Hurst. This simple species has a very local distribution in south-western North America and Mexico. It is certainly related to *Rosa* and also to *Potentilla* (Boulenger, 1936 *b*) and indicates long-existent polyphyletic lines of descent in the group.

There are three other diploid species all of which have been placed in monotypic genera by some taxonomists. Two of them, *Rosa microphylla* Roxb. (*Platyrhodon*) and *R. bracteata* Wendl. (*Ernestella*), are retained in the genus *Rosa* by Boulenger, each in a separate section. The third, *R. persica* Michx., he separates as *Hulthemia*, the generic name first proposed for it by Du Mortier (1824). It belongs to the deserts of Central Asia. It has simple, exstipulate leaves and densely prickly fruits. All these forms have probably been separated from the main line of descent in *Rosa* for long periods of time.

(2) *Hybridization in Pleistocene times*

Phenological studies in southern Michigan showed (Erlanson, 1930) that in early, mild springs the period of anthesis for individual bushes of early-flowering roses is longer and there is more overlapping between flowering periods of successive species; for it is the onset of the dry, hot July weather that stops flower production.

At the time of the greatest extension of the Pleistocene ice-sheet the mean annual temperature was only a few degrees lower than it is to-day in the regions affected. Any of the roses found in Michigan now could have thrived there then on unglaciated areas. At one time the ice-sheet extended all across Michigan as well as northern Illinois, Indiana and Ohio, and roses would have been confined to the southern borders of the latter states. During the Ice Age the days lengthened in spring in these regions just as they do now, but the spring temperatures would be lower until later in the year; a long spell of cool spring weather must have been the rule, and rose species

had a long flowering period with ample opportunities for interspecific hybridization. The same conditions would hold in Europe. Thus the ice-sheet tended to bring northern and southern species together both in space and in time. Chilling encourages the production of unreduced gametes, and it is reasonable to postulate the production in Pleistocene times of amphidiploids, tetraploids and of triploids in which duplication gave hexaploids. Crossings between tetraploid species followed by duplication may well have given rise to the octoploid *R. acicularis* as in octoploid *Aegilotricum* (Kihara & Katayama, 1931); or the hexaploid type crossed with a diploid species may have produced the octoploid.

When the Pleistocene ice-sheet was fully extended over Europe and America species belonging to the section Eglanteriae were no doubt to be found along its southern boundary. There is no reason to exclude the coexistence of primitive diploid and polyploid species, as well as more specialized diploid forms in warmer latitudes—the ancestors of the modern section Synstylae and other roses with southern distribution.

(3) *Phytogeography of certain roses*

The distribution of the hexaploid species in America is continuous and stretches from the Aleutian Islands and Alaska south to the northern border of California, to the mountains of Colorado and across from Alberta to northern New York (see Erlanson, 1929, Fig. 2). As I stated in 1929, "it would appear as though the hexaploid type has not arisen more than once, if at all on the American continent.... The hexaploid types of Cinnamomeae may have entered America from north-eastern Asia and spread south and east over the continent" (Erlanson, 1929, p. 482).

The greatest geographical range of a rose in America is that of *R. Woodsii* Lindl., a simple diploid form which stretches from the coast of Alaska to Chihuahua, Mexico. *R. blanda* Ait., which is related to *R. Woodsii* and gives fertile spontaneous hybrids with it, belongs to north-eastern North America. It extends from Pennsylvania to Anticosti, and to Illinois, the Dakotas, Manitoba and Hudson Bay (see Erlanson, 1929, Fig. 4). This is a diploid rose which can thrive under almost arctic conditions but it has not spread far to the south.

(4) *Generalized diploid types and polyploid relatives*

Both *R. blanda* and *R. Woodsii* are related to *R. cinnamomea* L. which is the most generalized and primitive diploid Eurasian species with a very extensive range. Boulenger, Crépin and also Rydberg were struck by the similarity between *R. cinnamomea*, *R. blanda* and *R. nutkana* Presl. The latter is a hexaploid species of north-western North America. Crépin placed *R. cinnamomea* and *R. nutkana* in the same group in the Cinnamomeae (Boulenger, 1936, p. 140). No one has ever suggested that diploid *R. cinnamomea* is descended from hexaploid *R. nutkana*, and phytogeography points to the opposite conclusion. Thus the simple diploid type, *R. cinnamomea* no doubt descended from a diploid ancestral type which may have given rise also to *R. Woodsii*, *R. blanda* and *R. nutkana* (hexaploid). The latter three species (combined perhaps with *R. rugosa* Thunb.) I consider to be ancestral to the tetraploids *R. Durandii* Crépin, and *R. californica* S. and C. on the Pacific Coast, to the tetraploid *R. arkansana* Porter of the Great Plains, and to *R. carolina* L. and *R. virginiana* Mill. of the north-eastern United States, as well as to the diploids *R. palustris* Marsh., *R. nitida* Willd. and *R. foliolosa* Nutt.

Several interspecific hybrids of varying degree of fertility have been obtained between members of the Cinnamomeae (Erlanson, 1934, Fig. 20). Unfortunately no studies of synapsis in the F_1, nor of comparative chromosome morphology in the genus, have yet been made. In an extensive study of the genus *Crepis*, Babcock (1934) and his co-workers find that the processes involved in the evolution of species are: (1) chromosome transformation, (2) amphidiploidy following interspecific hybridization, (3) autopolyploidy, (4) gene mutation. In *Rosa* all these processes have certainly also played important parts. Pairing behaviour indicates that translocation, reduplication of chromosome segments and the elimination of fragments frequently occur.

R. gymnocarpa Nutt. ranges from British Columbia to the hills of southern California. This rose often appears strikingly similar to some forms of *R. Woodsii*. It is a diploid like its close relative in Asia *R. Beggeriana* Schrenk. The latter is widespread in Asia between 30° and 50° latitude North at altitudes of 1500–5500 ft. (Boulenger, 1934). In Boulenger's opinion the fact that the styles, sepals and disk of the hypanthium are deciduous from the ripe fruit makes *R. gymnocarpa* and its Asiatic relatives "un petit group très naturel". He believes that they are related to the species in his section Pimpinelli-

Suavifoliae. The deciduous character could well be due to a single mutated gene, and although significant taxonomically it does not necessarily indicate much change phylogenetically.

R. acicularis (octoploid and hexaploid) is difficult to distinguish from *R. blanda* in regions where both of them grow. The hexaploid type gives semi-fertile tetraploid hybrids freely with *R. blanda*. *R. blanda* var. *hispida* Farwell is distinguished from *R. acicularis* by a larger inflorescence, longer flowering laterals, more stamens, a later flowering date and a longer fruit ripening period. The evolutionary tendency in *Rosa* is from simple to compound inflorescence, towards more numerous stamens and later flowering time.

(5) *Behaviour of polyploids and diploids compared*

When the relative advantages and disadvantages of the diploid and polyploid conditions are taken into consideration, particularly in a partially self-sterile genus, the hypothesis of parallel evolution of *R. acicularis* and *R. blanda* from a common ancestor is feasible.

The characteristics which distinguish *R. acicularis* and *R. blanda* may partly be due to the difference in their chromosome numbers. The physiological effect of polyploidy on the plant as a whole is usually to slow down the growth (Lindstrom, 1936); it may also confer greater adaptability as in polyploid *Dianthus* (Rohweder, 1934).

Among American rose species the higher polyploids respond at once to a slight rise in temperature and put forth foliage and flower buds very early in the spring. The European tetraploid *R. pimpinellifolia* also comes into flower precociously, but its diploid relatives *R. xanthina* Lindl. (syn. *R. Hugonis* Hemsl.) and *R. Primula* Boulenger flower synchronously with it or even earlier in America. Both Boulenger and I noticed that the more primitive species are the earliest flowering roses. They have short flowering laterals and a one- to few-flowered inflorescence. The polyploid condition which slows up growth would render it difficult for polyploid roses to flower and to mature fruit in boreal latitudes if they produced long flowering laterals. This primitive characteristic is thus selected by the environment. Hagerup (1932) found that polyploid species appear to be adapted to more extreme conditions of cold and drought than related diploids. In *Chrysanthemum* the decaploid species is apparently arctic.

A plant of octoploid *R. acicularis* from Alaska was transferred to

Ann Arbor, Michigan, where it leafed out soon after the first April thaw and was frequently badly damaged by frost in May. In some seasons all the floral primordial tissue was destroyed and no flowers were produced. The alternating thaws and frosts which are a feature of continental climates between 40 and 50° of latitude North, may militate against the spread southwards of octoploid races in *Rosa*. The Alaskan octoploid thrived at Pasadena (Erlanson, 1934) in southern California, and came into flower early in February, 3½ weeks before the hexaploid type.

It would seem as though polyploids are best adapted to arctic-alpine conditions. If the polyploids in *Rosa* were produced from unspecialized diploid species in Pleistocene times, as suggested above, they have remained in the habitat to which they are best suited by migrating northwards as the ice-sheet retreated.

Giant diploid grains occur as 0·3–2% of the pollen in some plants of *R. blanda* and *R. Woodsii* (Erlanson, 1934), but have not been observed in the more specialized diploid species. Autotriploids were found in diploid cultures of both these species (Erlanson, 1933). One culture of *R. blanda* contained a non-hybrid tetraploid as well as an autotriploid (Erlanson, 1934).

There are no reliable data on the genetics of *Rosa*. Allopolyploidy provides protected loci for gene mutations in new directions (Lindstrom, 1936). Yet it is almost impossible for a single autosomal recessive mutation to attain a homozygous condition and be selected out in a cross-breeding hexaploid or octoploid (Haldane, 1930). Dominant mutations cannot even be fully expressed when in the presence of four, five or more unmutated allelomorphs. Thus it is possible that the polyploid condition itself has been a bar to evolutionary progress for millennia in *Rosa*. However, types such as *R. acicularis* and *R. nutkana* may act as sources and reservoirs of new genes which are brought to light in the stable diploid descendants of unbalanced hybrids between them and diploid *R. blanda* and *R. Woodsii*. For example, *R. blanda* from northern Michigan was found to be extremely heterozygous and to throw seedlings which were tender 300 miles south of their natural habitat (Erlanson, 1929). The greatest degree of tolerance to different climates is shown by the simple diploid roses *R. blanda* and *R. Woodsii*. There are within *R. Woodsii* several distinct races which are adapted to specific environments as found in *Drosophila* by Timoféeff-Ressovsky (1936).

(6) *Physiological factors that limit range*

A factor which must be potent in limiting the southern boundary of boreal roses is the necessity for a period of after-ripening of the seeds at temperatures near 0° C. (Crocker, 1926). Hardiness, at least in some species, seems to be correlated with delayed development of the pro-embryo in the full-grown seed. Some races of *R. Woodsii*, as well as *R. rugosa*, do not exhibit this peculiarity, but this may have kept *R. blanda* and *R. acicularis* from spreading any farther south; under cultivation they thrive well anywhere in the United States.

R. nutkana from the State of Washington was unable to put out normal foliage and flower buds in the dry heat of southern California. The new growth was stunted and shrivelled and flowering was much delayed (Erlanson, 1934, Fig. 2). Other races of this species have done well in the moister atmosphere of Santa Barbara in Father Schoener's garden.

The more southern diploid American species such as *R. palustris*, *R. foliolosa* and *R. setigera* Michx. need a longer growing period before they flower, and for fruit ripening, than could be obtained far to the north. The two latter species are not viable much farther north than Detroit, Michigan.

SPECIALIZED TETRAPLOIDS

There is no primitive tetraploid species in America comparable to *R. pimpinellifolia* of Eurasia. The American tetraploid rose species fall into three distinct groups, each of which is more specialized in habitat preferences and inflorescence type than the related diploid form (Erlanson, 1929): (1) *R. carolina* and *R. virginiana* in the north-eastern region; (2) *R. arkansana* with a circumscribed area of distribution in the prairie region from Alberta and Saskatchewan south to Texas; (3) *R. californica* which is practically confined to California. All except *R. californica* are smaller than the related diploid. *R. arkansana* is semi-herbaceous and has a dwarf ecotype *R. alcea* Greene (Erlanson, 1934) which can withstand 60° below zero Fahrenheit in Canada (Wright, 1937). They are all plants of upland habitats as contrasted with the stream-bank and swamp habitats of the more primitive diploid and hexaploid species. The tetraploids come into flower after the related diploid species and usually continue to produce flowers in terminal corymbs on the season's turions throughout the season. *R. acicularis*, *R. blanda*, *R. nutkana* and *R. Woodsii* have a strictly limited flowering period of about 2 weeks,

In Eurasia there are specialized and highly evolved tetraploid roses represented by *R. gallica* L. (the Damask Roses) and some garden forms of *R. chinensis* Jacq. which are probably amphidiploids.

It is evident that tetraploid roses have arisen more than once on the American continent. These tetraploids probably arose from partially sterile diploid hybrids by duplication and appear to be of more recent origin than the higher polyploids and simple diploids (Erlanson, 1930). Their existence weakens the hypothesis of progressive evolution by descent of lower from higher chromosome numbers in *Rosa*.

SUMMARY AND CONCLUSIONS

Geographic distribution and cyto-taxonomy of *Rosa* together with data from the genetics and physiology of polyploids indicate that there have always been diploid lines of descent in the genus related to the polyploid lines.

Polyploidy is advantageous in arctic-alpine conditions, but it may slow up evolutionary changes. Physiological explanations for different types of range limitations are offered.

Hybridization of hexaploids with related diploids followed by the elimination of unpaired chromosomes in the descendants has no doubt been an important *modus operandi* in promoting the appearance of polymorphic diploid races with novel characteristics.

It is unnecessary to postulate a unique phylogenetic descent for modern rose species from a hypothetical arctic decaploid in order to account for cytological and phytogeographical conditions in the genus.

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REVIEW

Die Reliktföhrenwälder der Alpen. By EMIL SCHMID. Pflanzengeographische Kommission der Schweizerischen Naturforschenden Gesellschaft. Beiträge zur geobotanischen Landesaufnahme der Schweiz. Heft 21. H. Huber, Bern. 1936. 9.50 francs.

The pendulum swing of climate in geological time has played ducks and drakes with the flora and vegetation of Europe, bequeathing an intricate pattern varied as the geology, topography and soil. Southern elements hob-nob with northern, and while it is true to say that physical factors determine the survival of species in places with a specialized habitat—and so uneconomic as to escape destruction or exploitation by man—a full understanding of these alpine pinewoods comes only from a study of their historical and spatial relationships. Thus in this memoir a beginning is made with an account of the three floral regions which contribute to the floras of the pinewoods, the Mediterranean, the Irano-Turanian and the Euro-Siberian-North American. Each of these is divided into vegetation zones: the Mediterranean into the *Quercus ilex* and the *Q. pubescens* zones: the Irano-Turanian into the *Artemisia* semi-desert and the *Stipa* steppe: the Euro-Siberian-North American into the *Quercus robur-Calluna*, the *Lauroceraseus*, the *Quercus-Tilia-Acer* mixed wood, the *Pulsatilla* forest steppe, the *Fagus-Abies*, the *Picea*, the *Larix-Pinus cembra*, and the *Vaccinium uliginosum-Loiseleuria* zones.

The effect on the floras of changes in climate is discussed and a separation is made between the Transgression flora which overran the area of several vegetation zones exposed since the last ice age and the Inmutation flora of Southern Europe. The former is characterized by lack of stability and of sharp definition, the latter by a greater maturity, a better harmony with the environment and between the floristic components.

From the study of these floras and of the species proper to the different vegetation zones the relict pinewoods of the Alps are assigned, on the basis of their floristic composition, to their respective alliances. Eleven associations are described of which seven fall into the *Pinion silvestris* of the *Pulsatilla* forest steppe zone, and one each to the *Quercion pubescentis* of the *Q. pubescens* zone, the *Quercion-roboris acidiphilum* of the *Quercus robur-Calluna* zone, the mixed deciduous forest of the *Quercus-Tilia-Acer* zone, and one to the boundary between forest steppe and forest heath.

The habitats of these relict woods are described and generally speaking they are immature geomorphologically and in soil development with special microclimates and geological substrata. The species, in conformity with these facts, are generally neutrophilous or basiphilous.

Two folding maps show the horizontal and vertical distribution of the relict pinewoods and their spatial relationships to the relevant vegetation zones.

Pine is a fitting species to dominate these floristically varied types. Casting a light shade and tolerating a wide variety of soils and climates within the area of its distribution from the White Sea in the North to Southern Spain and from West Scotland far into Asia, it exhibits perhaps more than any other conifer a plasticity, expressed in its numerous races, which may account for its survival. This raises the question how far a possible lack of plasticity has sealed the fate of the gymnosperms and a corresponding variability has enabled the progressive angiosperms to dominate three-fifths of the earth's surface.

The memoir illustrates very clearly the usefulness of a knowledge of systematics and genetics in the interpretation of vegetation and the impossibility of attaining a full understanding of plant distribution and vegetation without taking the historical aspect into account. It is a tribute to the author and to Swiss ecologists, who realize the need for, and have the foresight to carry out, a proper ecological survey of their country.

A. S. WATT

SOCIETY FOR EXPERIMENTAL BIOLOGY

The Society for Experimental Biology holds three Conferences a year, at which papers and demonstrations are given on various aspects of experimental botany and zoology. Short accounts of the botanical proceedings appear in this *Journal*. Arrangements have been made whereby it is possible to obtain the *New Phytologist* through membership of the Society. Further particulars of the Society can be obtained from the Secretaries: J. Z. Young, Magdalen College, Oxford, and T. A. Bennet-Clark, University College, Nottingham.

The forty-first Conference of the Society was held at the London School of Hygiene and Tropical Medicine from 20 to 22 December 1937.

Two sessions were devoted to matters of botanical interest: papers on biochemical questions and on the mechanism of salt uptake were given in the first session, at which Professor Gregory took the Chair.

Miss Jones gave a paper on respiration and malates in succulents. She showed that there is an initial rhythmic fall and rise in respiration rate when *Kleinia* leaves are placed in darkness during summer, followed by a rise and fall resembling the climacteric which has been described for other tissues. There is finally a sudden outburst of CO_2 coinciding with the injection of the intercellular spaces and with a rapid final rise in the *p*H of the sap to 8–9. Changes in malate and calcium contents were followed.

Mr Burges gave an account of the pectic enzymes of *Sclerotinia*, which included a useful summary of the nomenclature of the pectic substances and their enzymes. Protopectinase, pectinase, lamellase (which hydrolyses middle-lamella pectin), and pectase (which converts pectin to pectin acid) were distinguished in part by their different heat sensitivities. Pectinase and lamellase are deactivated at 63° C. Soft rots like *Sclerotinia* contain all four enzymes, whereas leaf-spotters such as *Septoria* lack protopectinase and lamellase.

Dr Steward gave an account of the structure and life histories of three *Valonia* species, and described the conditions under which these grow at Fort Jefferson in the Tortugas. He emphasized that the natural variation in the composition of their sap is largely connected with exposure and light rather than with variation of the *p*H or CO_2 content of the sea water.

Mr Woodford described the method developed in Professor Gregory's laboratory for simultaneous determination of salt uptake and respiration rate of roots. He showed that the absorption of nitrate is increased by increase of the external concentration of salt and also by increase of oxygen tension. Even in the absence of an oxygen supply to the roots considerable absorption of nitrate occurs. Oxygen concentration had comparatively little effect on phosphate absorption, which proceeded as rapidly in air and nitrogen. These results were contrasted with those of Steward. In the discussion which followed Dr Steward emphasized that his work referred to accumulation of ions to a high internal concentration and stated his belief that nitrate and phosphate absorbed under anaerobic conditions must be converted into other substances rather than be accumulated as free ions.

Mr Harrison described the methods for quantitative determination of rubidium developed in conjunction with Dr Steward. Their data showed that the absorption of rubidium ions by potato disks is related to the specific surface and oxygen tension in a manner exactly parallel to that of bromide when RbBr is supplied. The importance of determining the absorption of both cation and anion indicators of absorption was emphasized.

Professor Skene took the Chair at the second session.

Professor Gregory and Mr Nutman described the development of the embryo in prematurely detached ears of rye. Complete but dwarf embryos are found even in grains detached as early as 5 days after fertilization. These produce normal plants on germination. The shoot primordia of all these dwarf embryos are about the same size though the embryo length is greater the longer the period of attachment.

Dr Tomkins discussed the nature of optimum growth rates as found in fungi, and the effects of inhibitors such as phenol on these optimum growth rates. Growth rates were expressed as change in diameter (of a circular colony) per unit time, and these rates remained constant for quite long times. Above the optimum temperature similar but much lower constant growth rates were found. In the discussion these constant growth rates were contrasted with the growth-time curves ordinarily found and it was suggested that the results were in part due to use of linear dimensions of the colony as a measure of growth.

Dr Pearse described work on the mode of action of root-forming substances. After treatment of the base of a cutting with indolyl- or naphthyl-acetic acid, and removal of the treated base, it was found that a second treatment of the cutting with indolyl-acetic acid did promote root formation. Cooper's claims that indolyl-acetic acid causes redistribution of the growth substance and that it itself does not act as a root-forming substance were thus not substantiated. Dr Pearse discussed the effect of ringing on the transport of the root-forming substance.

Miss Ernest described conditions affecting the fruiting of the higher fungi in culture. Those which do fruit, do so on a wide range of different media. In the case of the *Fomes* sp. dealt with in detail, fructifications were only formed after exposures of the mycelium to light. The requisite exposures were influenced by the glucose and peptone content of the medium.

Dr Goodall gave an account of work on the effects of light intensity and length of day on flowering of tomato. High light favours early flowering, and the optimum length of day is shorter the brighter the light: with bright lights it may be as short as 7 hours and with low light as long as 24. Plants sown in late autumn thus form their first flowers very late and those sown in spring flower at an earlier stage than those sown in summer. The stage of flowering is also affected by such factors as age of seed and size of first leaves.

Amongst exhibits of special interest to botanists were Professor Gregory's apparatus for studying salt uptake and respiration of roots, and the dwarf embryos from rye exhibited by Mr Nutman. Cultures of higher Basidiomycetes were shown by Miss Ernest. Dr Wigglesworth showed a simple method for carrying out micro-titrations, in which paraffined microburettes, etc., of 0·3 mm^3. capacity are used.

The discussion on Dollo's Principle in Evolution was attended by several botanists though they did not contribute to it.

T. A. B.-C.

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DISTRIBUTION OF CARBOHYDRATES BETWEEN COMPONENT PARTS OF THE WHEAT PLANT AT VARIOUS TIMES DURING THE SEASON

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(With 14 figures in the text)

INTRODUCTION

In a previous communication (Barnell, 1936) the drifts of various carbohydrates in wheat plants were studied for two varieties of wheat, Rivets and Wilhelmina, through two successive seasons. The interrelations of environmental factors and the underlying developmental drift were considered in some detail, and it was shown that during the winter and early spring months the sucrose content of the plant was, to a considerable extent, a function of the temperature; in later months a clear developmental drift of all the estimated carbohydrates was demonstrated. Differences between the two varieties in respect of their carbohydrate contents were practically negligible.

The work to be reported is a continuation of that briefly described above and consists of observations on the drifts of various carbohydrates in the component parts of the wheat plant during spring and summer.

Methods and procedure

The wheat, Rivets, was drilled on 16 October 1934 (seed rate: 2½ bushels per acre) in two plots[1] consisting of parallel strips of approximately half an acre in area. These were each divided into five subplots of approximately equal area. Sampling, as in 1933–4 (Barnell, 1936) consisted in removing a 1 ft. row unit sample at random from each subplot; the ten unit samples so obtained were grouped together to form a single sample and two such grouped samples were obtained from the plots at each sampling time. The samples were

[1] The two plots were separated by a ½-acre strip drilled with Wilhelmina wheat.

removed to the laboratory in closed containers and there the plants were divided into three groups according to their size. Two samples, each containing one-fifth the number of plants in the original two grouped samples, were finally obtained by taking one-fifth of the number of plants within each size-group. This reduction in size of the original samples was rendered necessary by the length of time required to divide the plants into their components and the necessity for reducing to a minimum the time between collecting the plants in the field and placing the parts for analysis into hot spirit. It was decided that a more representative sample of the plants in the plots was obtained by this method than by reducing the size of the sample taken from the field.

The plants from each reduced sample were then quickly separated by scalpels and scissors into leaves, leaf-sheaths, stems (from May onwards the stems were separated into (*a*) lower stems consisting of the bottom three internodes, (*b*) upper stems consisting of the remainder of the stem) and, when present, ears. These were weighed and placed in hot spirit (approximately 80%).

All samples were taken at sunrise to ensure, as nearly as possible, comparable conditions preceding each sampling. Sampling was carried out at monthly intervals from March to June and then two samples were gathered in July.

Analyses of carbohydrates were carried out as previously described (Barnell, 1936). Those estimated were: sucrose; glucose; fructose; polysaccharides as given by hydrolysis with taka-diastase, divided into fermentable (glucose) and non-fermentable (pentose) fractions; and glycosidic glucose. All carbohydrate fractions were estimated as reducing sugars, using the modified Shaffer-Hartmann copper method (Maskell) and the data presented in terms of glucose. No definitely positive iodine test showing the presence of true starch was obtained from any samples except those from the ears and that starch was present in the leaf, leaf-sheath and stem in the early morning only in traces, if at all, was confirmed in some cases by the use of β-malt amylase in the starch determination method described by Hanes (1936).

In addition dry matter determinations were made with subsamples of each sample and estimates of the total alcohol soluble substances obtained. Fructosans have been reported present in wheat (Belval, 1924), in barley leaves (Archbold, 1935; Yemm, 1935), and in various grasses (Cugnac, 1931), accordingly an attempt has been made to follow the course of their amounts in the various plant parts in the present work. The method adopted was to extract the

alcohol-extracted residue with water for 48 hours, filter and make up to volume.

The extract[1] was boiled for 10 min. with $N/5$ HCl, cooled, neutralized and made up to volume. The total reducing value was determined by the copper method and then the keto-reducing value after oxidation by hypo-iodite.

Weather

The 1935 season at Cambridge, from March to harvest, was dry (less than 7 in. of rain fell during the five months March to July), though not quite so dry as the preceding season but drier than the corresponding period in 1933. In respect of sunshine the 1935 season occupied an intermediate position between the 1933 and 1934 seasons (for the five months, March to July, the hours of sun were: 1933, 990; 1934, 859; 1935, 912).

In the present work only the broad developmental drifts of carbohydrates within the plant have been studied and not the immediate reactions of the carbohydrate interrelations to fluctuating environmental factors.

PROPORTIONS OF LEAF, LEAF-SHEATH, STEM AND EAR TISSUE PRESENT AT VARIOUS TIMES IN THE WHEAT PLANT

The relative amounts of leaf, leaf-sheath, stem and ear tissues changed considerably during the season; the course of the percentage amount of each relative to the weight of the whole plant (fresh weights) during 1935 is shown in Fig. 1. The proportion of leaf tissue fell during the period investigated from over 70% in March to less than 10% in July. The leaf-sheath tissue rose from 20% in March to nearly 30% in April and changed little till June, subsequently falling in its July values. In March the stem tissue constituted less than 10% of the plant but rose during the following months reaching 59·7% on 5 July but then falling to 45·5% on 24 July. The proportion of ear tissue to the fresh weight of the whole plant rose from 2·3% on 8 June to 36·5% on 24 July.

DRIFTS OF THE PERCENTAGE AMOUNTS OF DRY MATTER AND VARIOUS CARBOHYDRATES IN THE COMPONENT PARTS OF THE WHEAT PLANT

Dry matter

Dry-matter contents are set out in Fig. 2 for each component part of the wheat plant and for the whole plant (calculated from the

[1] Progress curves for fructose yield from wheat fructosans against time of boiling with $N/5$ HCl show that the peak value is obtained after 10 min. but it is very sharply defined; more consistent values are obtained by hydrolysis with $N/5$ HCl for 25 min. at 60° C.

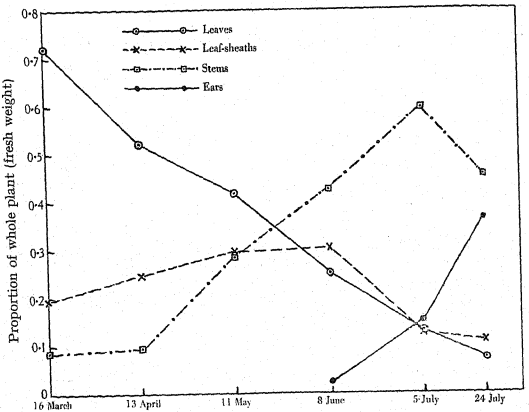

Fig. 1. Ratios of fresh weights of leaves, leaf-sheaths, stems and ears respectively, to the fresh weight of the whole plant on various sampling dates.

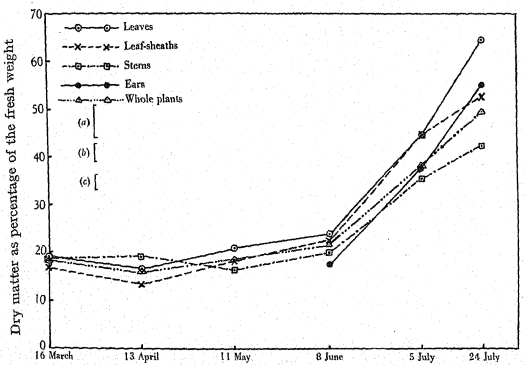

Fig. 2. Dry-matter contents of whole plants, leaves, leaf-sheaths, stems and ears of Rivets wheat. The magnitudes of differences which correspond to $P=0{\cdot}05$ level of significance are shown as vertical lines: (*a*) for leaves, (*b*) for leaf-sheaths, and (*c*) for stems.

values of its components) for each sampling date from March to July.

The dry-matter content showed a definite drift within each component; in the leaves and leaf-sheaths it began to rise slowly from mid-April and then rapidly from early June; in the stem the rise did not begin until June. During the period following ear-emergence, early June, all parts of the plant lost water rapidly—particularly leaves and leaf-sheaths—the dry-matter content of the leaves rising to 64·4% on 24 July, a few days before harvest; the stem contained 42·1% dry matter on the same date and the ears 55·1%. The drift of percentage dry matter in the whole plant followed that of the leaves and leaf-sheaths fairly closely from March to June and then, as the stem and ear tissues increased in proportion to the other components it followed these more closely.

Total alcohol soluble substances, total sugars and non-sugars in leaves

The total alcohol soluble substances shown in Fig. 3, expressed as percentages of the fresh weight, fell from 16 March to 13 April and then rose to 5 July, falling to 24 July; the non-sugar fraction followed a similar course but the total sugars fell continuously apart from a small rise on 5 July.

The effect of the underlying drift in the water content of the leaves is sufficient to cause the amounts of each fraction to show falling values throughout the period when expressed as percentages of the dry weight (Table I); and continuous rising values from 13 April in the total alcohol soluble substances and non-sugar fraction when expressed as concentrations, i.e. percentages of the water content.

TABLE I. *Expression of data as percentages of fresh weight, dry weight and of water content*

	Total alcohol soluble substances			Non-sugars			Total sugars		
Sampling date	As % of fresh weight	As % of dry weight	As % of water	As % of fresh weight	As % of dry weight	As % of water	As % of fresh weight	As % of dry weight	As % of water
16 Mar.	5·64	28·4	6·98	4·01	20·9	4·96	1·63	8·51	2·02
13 Apr.	4·32	25·7	5·19	2·93	17·5	3·52	1·39	8·25	1·67
11 May	5·12	24·5	6·47	4·26	20·4	5·38	0·86	4·13	1·09
8 June	5·20	21·8	6·84	4·59	19·2	6·03	0·61	2·65	0·80
5 July	6·49	14·5	11·8	5·66	12·7	10·3	0·83	1·86	1·51
24 July	4·92	7·65	13·8	4·66	7·25	13·1	0·26	0·41	0·73

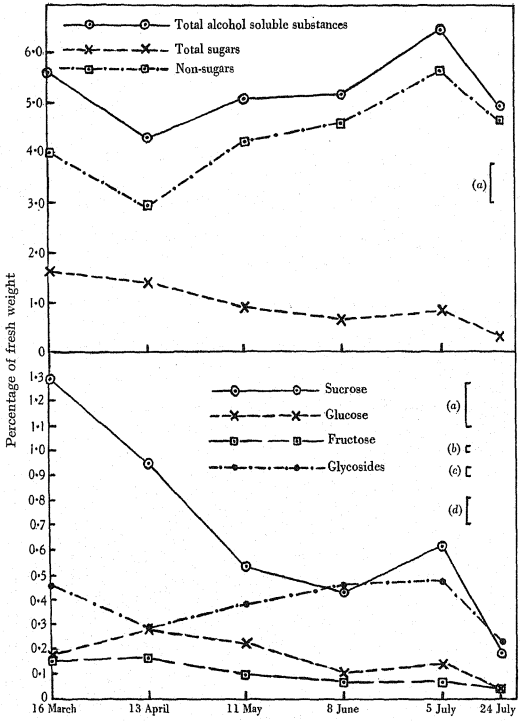

Fig. 3. Top curves show drifts of total alcohol soluble substances and the component fractions, total sugars and non-sugars in the leaves. Lower curves give the drifts of sucrose, glucose, fructose and glycoside glucose in the leaves. The minimum significant difference is given for the total alcohol soluble substances by the vertical line labelled (*a*) in the top curves and for (*a*) sucrose, (*b*) glucose, (*c*) fructose, (*d*) glycoside glucose by those labelled (*a*), (*b*), (*c*), (*d*) in the lower curves.

Sucrose, glucose, fructose and glycosidic glucose in leaves

The most striking feature of the drifts of the sugars in the leaves shown in Fig. 3 is the continuous fall in the percentage amount of sucrose from March onwards, a peak value probably having occurred earlier than the first sampling date. The rise in the sucrose between 8 June and 5 July is barely significant, so must be regarded as of subsidiary importance, though this was the date on which sucrose was at its maximum concentration in the plant as a whole. Glucose showed a peak value on 13 April, but the rise between 8 June and 5 July, though small, was significant. Fructose, present in less concentration than glucose, fell practically continuously throughout the period: the final concentration on 24 July was equal to that of glucose. Glycosidic glucose expressed as percentage of the fresh weight showed a rise from 13 April to 5 July, but the amount relative to dry matter was actually falling slightly; the fall to 24 July was large enough to show on the fresh weight basis of presentation of data and coincided with the period during which utilization of sugars in starch formation in the ears was vigorous.

Taka-diastase hydrolysis products in leaves

Little change occurred in the percentage of taka-diastase hydrolysis products in the leaves during the period investigated. The drift plotted in Fig. 4 showed a rise from April to May and the higher level was maintained during June and then fell to 5 July, rising again to 24 July. The non-fermentable (pentose) fraction was present in greater amount than the fermentable (glucose) fraction after March and showed little variation throughout the period. The amount of this fraction relative to dry matter[1] in the leaves was falling continuously from April onwards, the drift of water contents masking this trend in the data expressed on the fresh weight basis. The drift of the fermentable fraction followed an approximately similar course when expressed as percentage of fresh weight (Fig. 4) or as percentage of dry weight; the significant fall in this fraction between 8 June and 5 July coincides with the inception of condensation of sugars to starch in the ears.

[1] Mean values for the non-fermentable and fermentable fractions of the taka-diastase hydrolysis products expressed as percentages of the dry weight were:

	16 Mar.	13 Apr.	11 May	8 June	5 July	24 July
Non-fermentable	1·40	2·07	1·99	1·58	0·64	0·29
Fermentable	1·62	1·29	1·44	1·34	0·27	0·41

Total alcohol soluble substances, total sugars and non-sugars in leaf-sheaths

The drifts of the total alcohol soluble substances in the leaf-sheaths shown in Fig. 5 was similar to that in the leaves except that the peak value was attained on 8 June instead of 5 July. The percentage amount of the non-sugar fraction, after falling slightly between March and April, rose slowly from April to June and then increased considerably between 8 June and 5 July subsequently falling slightly at the final sampling date. The total sugars showed a conspicuous maximum value on 8 June.

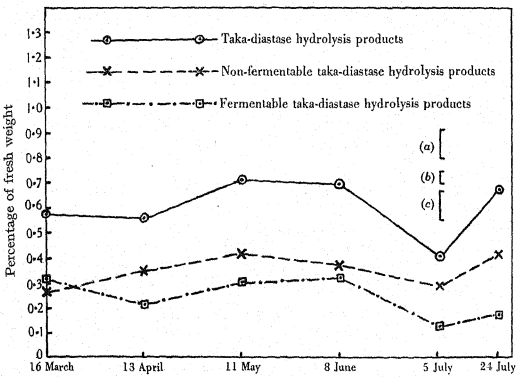

Fig. 4. Drifts of taka-diastase hydrolysis products and the component fermentable and non-fermentable fractions in the leaves. The vertical lines labelled (*a*), (*b*), (*c*) indicate the minimum significant differences, (*a*) of total taka-diastase hydrolysis products, (*b*) of non-fermentable taka-diastase hydrolysis products, (*c*) of fermentable taka-diastase hydrolysis products.

Sucrose, glucose, fructose and glycosidic glucose in leaf-sheaths

The drifts of the above sugars are plotted in Fig. 5. All three sugars and the glycosidic glucose attained peak values on 8 June. The high value of the sucrose percentage in March was probably caused, to some extent at least, by the low temperatures prevailing during the week preceding sampling (mean minimum temperature

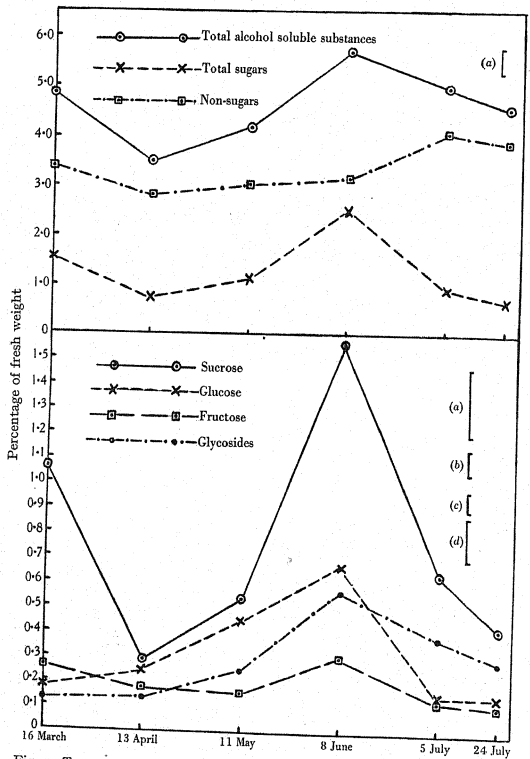

Fig. 5. Top curves show drifts of total alcohol soluble substances and the component fractions, total sugars and non-sugars in the leaf-sheaths. Lower curves give the drifts of sucrose, glucose, fructose and glycoside glucose in the leaf-sheaths. The minimum significant difference is given for the total alcohol soluble substances by the vertical line labelled (*a*) in the top curves and for (*a*) sucrose, (*b*) glucose, (*c*) fructose, (*d*) glycoside glucose by those labelled (*a*), (*b*), (*c*), (*d*) in the lower curves.

during preceding week = 31·1° F.). The percentage amount of glucose rose steadily from March to the peak value on 8 June and then fell, changing little between 5 and 24 July. Fructose fell from March to May and then rose to its peak value on 8 June, subsequently falling to 5 July and then slightly to 24 July. The glycosidic glucose showed little change till May and then rose to 8 June, afterwards falling continuously to 24 July.

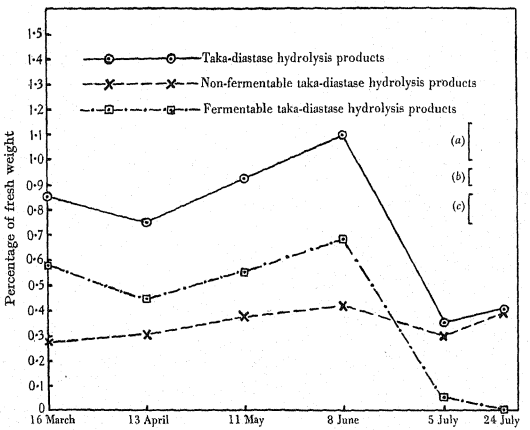

Fig. 6. Drifts of taka-diastase hydrolysis products and the component fermentable and non-fermentable fractions in the leaf-sheaths. The vertical lines labelled (*a*), (*b*), (*c*) indicate the minimum significant differences, (*a*) of total taka-diastase hydrolysis products, (*b*) of non-fermentable taka-diastase hydrolysis products, (*c*) of fermentable taka-diastase hydrolysis products.

Taka-diastase hydrolysis products in leaf-sheaths

The outstanding feature of the drifts shown in Fig. 6 is the fall to zero value of the fermentable fraction of the hydrolysed polysaccharides on 24 July. The fermentable fraction was present in greater amount than the non-fermentable from March until July, rising steadily from April until 8 June, then falling to a low value on 5 July and not detectably present on 24 July.

The relatively high proportion of this fraction present during the early months of the season suggests that it is derived from a temporary carbohydrate reserve readily hydrolysable to glucose. The fall in the amount of this fraction during July coincides with the utilization of sugars for starch formation in the ears.

Little change occurred in the amount of the non-fermentable fraction expressed as a percentage of the fresh weight throughout the period investigated, but the percentage amount relative to the dry matter of the plant tissue fell from June onwards.

Total alcohol soluble substances, total sugars and non-sugars in stems

The amounts of total alcohol soluble substances in the stem (plotted in Fig. 7) during March and April were lower than in the leaves and leaf-sheaths but later rose to much higher values. There was a significant fall in the amount of total alcohol soluble substances between March and April, but after April a steady rise occurred to a peak value attained on 5 July. The total sugars component followed a similar course over the period. The non-sugar alcohol soluble content was less than in the leaves throughout the whole period. It showed a steady rise from April till the final sampling date.

Sucrose, glucose, fructose and glycosidic glucose in the stems

The drifts of the free sugars and of glycosidic glucose in the stem are shown in Fig. 7. Sucrose, after falling from its relatively high value on 16 March, rose steadily from 13 April to a high maximum value of 4·53% of the fresh weight on 5 July, followed by a sharp fall to 24 July, coinciding with the period of starch accumulation in the ears. Glucose increased from April, attaining its peak value on 8 June, and then fell throughout July. Fructose rose steadily from March onwards (the slight fall shown between 8 June and 5 July was not significant), rising above the glucose content in July. The glycoside glucose content rose steadily from a very low value in March to a peak value on 5 July, the subsequent slight fall to 24 July was not significant.

Taka-diastase hydrolysis products in stems

The total taka-diastase hydrolysis products and the component fractions (shown in Fig. 8) rose to peak values on 11 May and then fell; but in the non-fermentable fraction and in the total hydrolysis

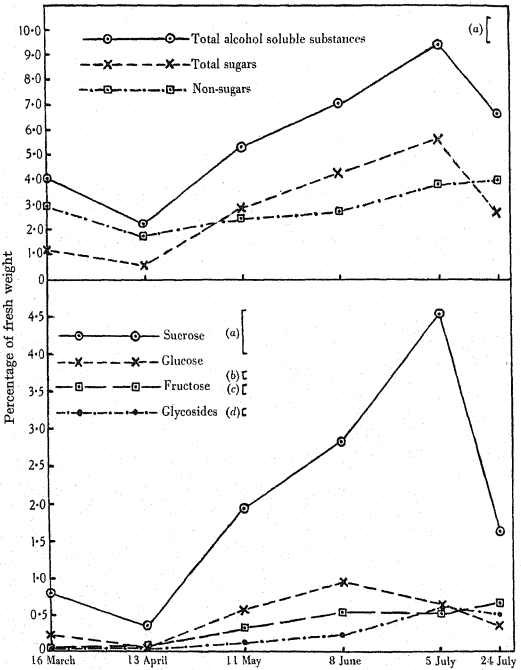

Fig. 7. Top curves show drifts of total alcohol soluble substances and the component fractions, total sugars and non-sugars in the stems. Lower curves give the drifts of sucrose, glucose, fructose and glycoside glucose in the stems. The minimum significant difference is given for the total alcohol soluble substances by the vertical line labelled (*a*) in the top curves and for (*a*) sucrose, (*b*) glucose, (*c*) fructose, (*d*) glycoside glucose by those labelled (*a*), (*b*), (*c*), (*d*) in the lower curves.

products a rise followed between 5 and 24 July. The fermentable fraction was absent on the March sampling date but was present in normal amount in April, increased slightly to 11 May and then fell after 8 June, changing little between 5 and 24 July.

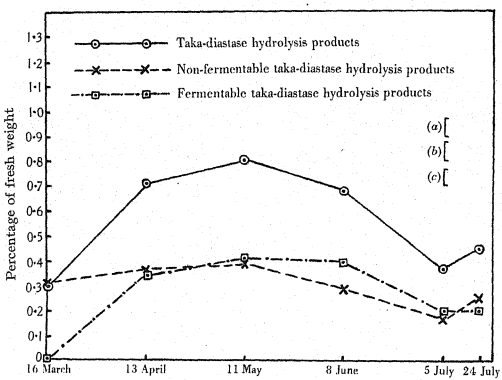

Fig. 8. Drifts of taka-diastase hydrolysis products and the component fermentable and non-fermentable fractions in the stems. The vertical lines labelled (*a*), (*b*), (*c*) indicate the minimum significant differences, (*a*) of total taka-diastase hydrolysis products, (*b*) of non-fermentable taka-diastase hydrolysis products, (*c*) of fermentable taka-diastase hydrolysis products.

Changes in the ears during June and July

The changes in the ears are not presented in graphical form since samples of ears were collected on three occasions only. Table II sets out the mean values of each estimated substance for each sampling date.

TABLE II. *Carbohydrate drifts in the ears (expressed as percentages of the fresh weight)*

Date	Dry matter	Total alcohol soluble substances	Glucose	Fructose	Sucrose	Glycosides	Taka-diastase hydrolysis products	Fermentable taka-diastase hydrolysis products	Non-fermentable taka-diastase hydrolysis products
8 June	17·57	5·50	0·311	0·148	1·570	0·014	1·398	0·814	0·584
5 July	37·6	7·70	0·460	0·266	2·841	0·230	2·651	2·137	0·515
24 July	55·1	2·92	0·055	0·056	0·552	0·068	22·465	20·769	1·699

The percentage of dry matter in the ear increased from 8 June (17·57%) to the last sampling date when it constituted 55·1% of the fresh weight. The total alcohol soluble substances, the glycosidic glucose and each of the three sugars, rose to maximum values on 5 July and then fell to 24 July. The percentage of taka-diastase hydrolysis products in the ear was approximately doubled between 8 June and 5 July and then increased tenfold to 24 July, the major part of the increase being due to the fermentable fraction containing glucose from hydrolysed starch. The non-fermentable fraction, however, increased to about three times its value of 5 July by the same date.

Fructosans in leaves, leaf-sheaths, stems and ears

Table III sets out for each plant component on each sampling date the amounts (as percentages of the fresh weight) of the keto-reducing substance (fructose) estimated after hydrolysis and hypo-iodite oxidation of the water extract of alcohol-extracted plant tissues.

TABLE III. *Fructosans in leaf, leaf-sheath, stems and ears (expressed as percentages of the fresh weight)*

Date	Leaves	Leaf-sheaths	Whole stems	Upper stems	Lower stems	Ears
16 Mar.	0·214	0·522	0·378	—	—	—
13 Apr.	0·073	0·216	0·139	—	—	—
11 May	0·125	0·263	0·286	0·203	0·343	—
8 June	0·215	0·690	0·220	0·135	0·299	3·021
5 July	0·109	0·204	0·196	0·215	0·173	0·913
24 July	0·168	0·119	0·155	0·192	0·107	0·293
Sig. diff. ($P=0·05$)	(0·098)	(0·098)	(0·064)			

These values are shown plotted in Fig. 9. The fructosan content of the leaves showed little change during the season, the small fluctuations being barely significant. In the leaf-sheaths there was a relatively high value in March and then a fairly high peak value (0·690%) on 8 June, the same time as very high amounts were present in the ears. The fructosan content of the stems as a whole was fairly high in March, falling to April, but rising again in May and then falling to the end of July. In Table III, columns five and six give values for the fructosan content of the upper and lower parts of the stem separately, and these show that during May and June the lower stem had a higher fructosan content than the upper, but during July conditions were reversed, suggesting, perhaps, that

fructosans are transitory carbohydrate reserves, deposited during the translocation of sugars to the developing ears.

The most interesting fructosan values are those for the ears. The amount of fructosans in the young ears on 8 June was very high (3·02 %) but fell quickly as the ears matured. These results are in accord with those of Belval (1924) and support his view that fructose-yielding polysaccharides play an important part as a transitory carbohydrate reserve, particularly in the young ears.

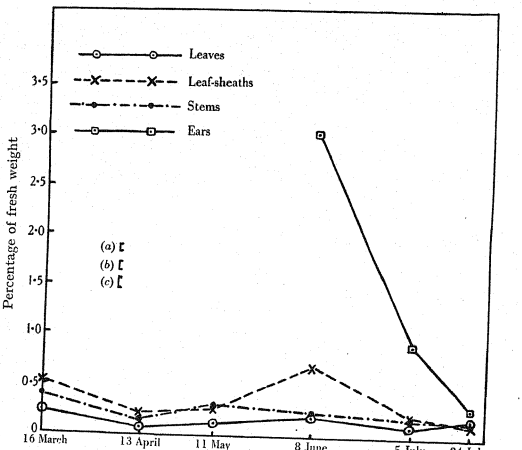

Fig. 9. Drifts of fructosans in the leaves, leaf-sheaths, stems and ears. The vertical lines labelled (*a*), (*b*), (*c*) give the minimum significant differences in (*a*) stems, (*b*) leaf-sheaths, (*c*) leaves.

Simultaneous changes in ear, upper and lower stem, leaf-sheath and leaf

The means of duplicate samples for each substance estimated, except fructosans, are set out for each plant component in Table IV for each of the sampling dates on which the ears were separately analysed.

The percentage of dry matter in all tissues rose from 8 June to 24 July. Total alcohol soluble substances rose in both upper and

TABLE IV. *Simultaneous changes in the carbohydrates of the leaf, leaf-sheath, stem and ear (expressed as percentages of the fresh weight)*

Sampling date	Leaf	Leaf-sheath	Stem			Ear
			Upper	Lower	Whole	
Dry matter:						
8 June	23·96	22·24	17·56	22·09	19·90	17·57
5 July	44·8	44·8	36·2	34·0	35·2	37·6
24 July	64·35	52·7	41·6	42·8	42·1	55·1
Total alcohol soluble substances:						
8 June	5·20	5·72	6·38	7·75	7·08	5·50
5 July	6·49	4·99	9·11	9·88	9·47	7·70
24 July	4·92	4·55	6·34	6·60	6·61	2·92
Glucose:						
8 June	0·104	0·670	1·037	0·807	0·920	0·311
5 July	0·140	0·151	0·466	0·815	0·624	0·460
24 July	0·037	0·143	0·281	0·422	0·342	0·055
Fructose:						
8 June	0·069	0·302	0·563	0·551	0·556	0·148
5 July	0·068	0·130	0·282	0·773	0·502	0·266
24 July	0·038	0·107	0·545	0·813	0·661	0·056
Sucrose:						
8 June	0·436	1·567	2·242	3·378	2·824	1·570
5 July	0·624	0·639	4·628	4·402	4·527	2·841
24 July	0·187	0·427	1·575	1·675	1·620	0·552
Fermentable taka-diastase hydrolysis products:						
8 June	0·321	0·688	0·362	0·432	0·393	0·814
5 July	0·120	0·056	0·268	0·118	0·199	2·137
24 July	0·263	0·006	0·257	0·111	0·194	20·767
Non-fermentable taka-diastase hydrolysis products:						
8 June	0·377	0·416	0·302	0·279	0·290	0·584
5 July	0·287	0·301	0·222	0·094	0·165	0·515
24 July	0·415	0·401	0·212	0·303	0·252	1·699
Glycosides:						
8 June	0·456	0·569	0·282	0·171	0·225	0·014
5 July	0·474	0·386	0·594	0·571	0·582	0·230
24 July	0·230	0·283	0·478	0·527	0·500	0·068

lower stem and ear from 8 June to 5 July and then fell to 24 July. A small rise occurred also in the leaf tissue between the same dates, but the rise and fall in the stem (Fig. 7) and ear was mainly due to sugars, while that in the leaf (Fig. 3) was mainly due to non-sugars. The percentage amount of glucose fell considerably in the leaf-sheath and upper stem tissues between 8 June and 5 July, while small increases occurred in the leaf, lower stem and ear tissues; in all tissues the amount present fell between 5 and 24 July. Fructose was the only sugar which accumulated in any tissue between 5 and 24 July; it showed definite increases in percentages of the fresh weight in both upper and lower stems while, simultaneously, in the same tissues fructosans were decreasing in amount (Table III). Between

the same dates fructose fell in amount in the leaf-sheath and ear, though in the ear it had previously increased between 8 June and 5 July.

On any one date, sucrose was present in greatest concentration in the stem; then, in order of concentrations come the ear, leaf-sheath and leaf tissues. In general, one can say that the lower stem had a higher concentration of sucrose than the upper stem and the upper stem a higher concentration than the ear. So if similar relations hold in the postulated channels of transport, the sieve-tubes, movement of sucrose up the stem into the ear would be along a positive concentration gradient. The leaves and leaf-sheaths at this late stage of the season probably play little part in the formation of carbohydrates, photosynthesis being mainly carried out by the green stems, but even so it is rather surprising to find a negative concentration gradient from the leaves to the stem.

The glycoside glucose in the leaf-sheath fell from 8 June to 24 July, but in each of the other tissues it rose from 8 June to 5 July and then fell. The fermentable (glucose) fraction of the taka-diastase hydrolysis products accumulated to a high percentage in the ear, particularly during the period between 5 and 24 July, while in all other tissues (apart from the leaf between 5 and 24 July) it fell. The non-fermentable (pentose) fraction accumulated irregularly to some extent in each tissue but particularly in the ear.

Total amounts of dry matter and of various carbohydrates in the component parts of rivets wheat during 1935

In a previous communication (Barnell, 1936) reasons were given for presenting data as amounts of each substance per 10 ft. row rather than expressing them as amounts per plant or per tiller. The same practice has been adopted with the present material, the data for each estimated substance being given as amounts per 10 ft. row in leaves, leaf-sheaths, stems (upper, lower and whole), ears and whole plants in Table V.

The total amounts per 10 ft. row of dry matter in whole plants and the component parts are plotted in Fig. 10 against the sampling dates. The dry weight per 10 ft. row of aerial plant tissue followed a normal growth curve during the period investigated, increasing slowly during March and April and then more rapidly from April onwards, slowing down between 5 and 24 July when the ears were ripening and all tissues except ears were losing weight. The total

TABLE V. *Total amounts in g. per 10 ft. row*

Date	Dry matter	Total alcohol soluble substances	Glucose	Fructose	Sucrose	Glyco-sides	Taka-diastase hydrolysis products	Ferment-able taka-diastase hydrolysis products	Non-ferment-able taka-diastase hydrolysis products	Fructo-sans
Leaves:										
16 Mar.	30·3	8·97	0·321	0·233	2·036	0·723	0·916	0·491	0·425	0·333
13 Apr.	48·8	12·83	0·702	0·811	2·761	0·864	1·640	0·627	1·013	0·305
11 May	92·4	22·56	1·007	0·426	2·400	1·719	3·160	1·320	1·841	0·564
8 June	120·0	26·01	0·517	0·339	2·280	2·288	3·500	1·610	1·892	1·075
5 July	127·0	18·51	0·390	0·189	1·758	1·349	1·155	0·335	0·820	0·325
24 July	88·0	6·79	0·051	0·046	0·255	0·330	0·918	0·333	0·585	0·228
Leaf-sheaths:										
16 Mar.	7·2	2·10	0·078	0·110	0·462	0·055	0·366	0·248	0·118	0·280
13 Apr.	18·2	4·82	0·335	0·236	0·403	0·184	1·045	0·619	0·426	0·283
11 May	56·5	12·95	1·391	0·481	1·664	0·748	2·920	1·740	1·180	0·825
8 June	135·0	34·39	4·030	1·810	9·390	3·424	6·690	4·170	2·518	4·160
5 July	123·0	13·57	0·400	0·395	1·696	1·042	0·977	0·151	0·826	0·548
24 July	111·0	9·55	0·305	0·217	0·904	0·618	0·854	0·027	0·827	0·253
Stems:										
16 Mar.	3·6	0·78	0·044	0·011	0·158	0·008	0·056	0·000	0·056	0·103
13 Apr.	10·1	2·17	0·034	0·043	0·188	0·024	0·372	0·181	0·191	0·070
11 May	46·8	15·23	1·631	0·919	5·590	0·383	2·320	1·180	1·140	0·864
8 June	170·0	60·40	7·840	4·740	24·000	1·918	5·820	3·350	2·470	1·878
5 July	462·0	124·30	8·190	6·600	59·400	7·640	4·780	2·620	2·160	2·696
24 July	368·0	57·80	2·990	5·770	14·150	4·360	3·880	1·680	2·200	1·186
Upper stems:										
16 Mar.	—	—	—	—	—	—	—	—	—	—
13 Apr.	—	—	—	—	—	—	—	—	—	—
11 May	19·9	6·72	0·720	0·385	2·151	0·131	0·928	0·423	0·505	0·252
8 June	71·0	25·83	4·230	2·300	9·100	1·140	2·710	1·480	1·230	0·544
5 July	261·0	65·60	3·360	2·010	33·080	4·300	3·520	1·920	1·600	1·580
24 July	208·0	32·76	1·405	2·790	7·950	2·300	2·355	1·291	1·065	0·814
Lower stems:										
16 Mar.	—	—	—	—	—	—	—	—	—	—
13 Apr.	—	—	—	—	—	—	—	—	—	—
11 May	29·2	9·70	0·999	0·584	3·820	0·278	1·541	0·850	0·691	0·612
8 June	99·0	34·52	3·620	2·490	15·090	0·742	3·120	1·720	1·395	1·334
5 July	201·0	63·75	5·260	4·950	28·410	3·681	1·368	0·762	0·606	1·116
24 July	160·0	24·45	1·552	2·910	6·030	2·020	1·536	0·440	1·096	0·372
Ears:										
8 June	8·0	2·53	0·143	0·067	0·719	0·007	0·645	0·376	0·269	1·417
5 July	125·0	25·54	1·520	0·880	9·410	0·783	8·850	7·140	1·713	3·030
24 July	385·0	20·30	0·396	0·378	3·870	0·478	156·700	144·910	11·740	2·110
Whole plant:										
16 Mar.	41·1	11·85	0·443	0·357	2·656	0·786	1·340	0·739	0·600	0·717
13 Apr.	77·1	19·82	1·161	1·090	3·352	1·072	3·057	1·427	1·630	0·558
11 May	195·7	50·74	4·029	1·826	9·654	2·850	8·400	4·240	4·164	2·253
8 June	433·0	123·33	12·530	6·960	36·380	7·640	16·660	9·510	7·150	8·530
5 July	837·0	181·92	10·490	8·020	72·260	10·810	15·760	10·246	5·520	6·599
24 July	952·0	94·44	3·740	6·410	19·180	5·786	162·350	146·950	15·350	3·777

amounts of dry matter per 10 ft. row in each of the component parts of the plant are shown in the same figure. The dry matter in leaf tissue increased continuously from March till 5 July (although the percentage amount of leaf tissue relative to the whole plant on either the fresh or dry weight basis, Fig. 1, fell continuously from March to the end of the season). The dry matter per 10 ft. row in leaf-sheath

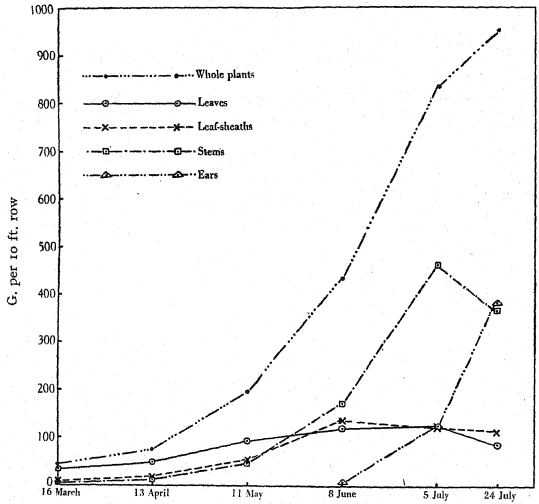

Fig. 10. Progress of dry matter accumulation in the whole plant, leaf, leaf-sheath, stem and ear respectively per 10 ft. row of wheat during 1935.

tissue was very small in March and April but increased during May, reaching its maximum amount on the 8 June sampling date, falling on each of the following two samplings. The amount in stem tissue increased slowly during March and April, then followed a course similar to the growth curve of the whole plant till 5 July but fell between 5 and 24 July. The 8 g. of visible ear tissue on 8 June increased to 385 g. during the $6\frac{1}{2}$ weeks to 24 July. All other tissues

and particularly the stem tissue lost weight during this interval. During this last phase of the plant's development the values of the critical concentrations of sugars for starch formation in the ear must have been so low that, not only did the products of photosynthesis move into the ear as formed in the shoot, but the carbohydrate constituents of each of the other tissues were broken down and transported in some form into the ears.

The data set out in Table V are shown as drifts of the various carbohydrates during the season in Figs. 11, 12 and 13 for leaves, leaf-sheaths and stems (for ears see Table V) respectively, while in Fig. 14 the drifts of fructosan amounts are shown for each tissue.

Each tissue behaved similarly to the others in regard to its total content of a particular carbohydrate but with differing time-relations between the tissues, e.g. sucrose rose to a peak value in the leaf on 13 April and then fell throughout the rest of the season, the peak value for sucrose in the leaf-sheath was not attained until 8 June, while in the stem and ear it was still later, 5 July. To varying extents this was true of each of the other carbohydrate constituents:

Dates of attaining maximum amounts of various carbohydrates in each tissue

	Total dry matter	Sucrose	Glucose	Glyco-side glucose	Fruc-tose	Fruc-tosans	Ferment-able taka-diastase hydrolysis products	Non-fer-mentable taka-diastase hydrolysis products
Leaf	5 July	13 Apr.	11 May	8 June	13 Apr.	8 June	8 June	8 June
Leaf-sheath	8 June	8 June	8 June	8 June	8 June	8 June	8 June	8 June
Stem	5 July	5 July	5 July	5 July	5 July	5 July	8 June	8 June
Ear	24 July	5 July	5 July	5 July	5 July	5 July	24 July	24 July

In the leaf tissues all total amounts of estimated carbohydrates attained and passed their maximum amounts before the dry weight total of leaf per 10 ft. row was maximal. The maximum dry weight of the leaf-sheath tissue was attained on 8 June, the same date as that for all the constituent estimated carbohydrates. In the stem[1] tissue all the soluble carbohydrates attained their maximum amounts on the same date as the dry weight of stem tissue (5 July), but the

[1] In lower stem tissue the dates for the peak values of the reserve carbohydrates (8 June) and the soluble carbohydrates (5 July) were the same as for the whole stems except that fructosans were maximal on 8 June instead of 5 July. In the upper stem glucose and fructose were maximal on 8 June and 24 July respectively, taka-diastase hydrolysis products on 5 July (see Table V).

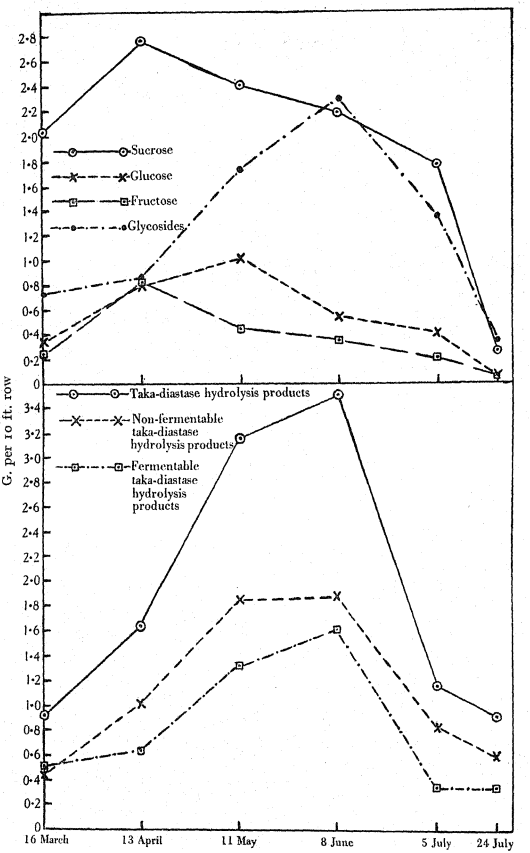

Fig. 11. Progress of carbohydrate accumulation in the leaves. The amounts of sucrose, glucose, fructose and glycoside glucose present in the leaves at various times are shown in the top curves. The lower curves show the amounts of taka-diastase hydrolysis products, non-fermentable taka-diastase hydrolysis products and fermentable taka-diastase hydrolysis products present at the same times.

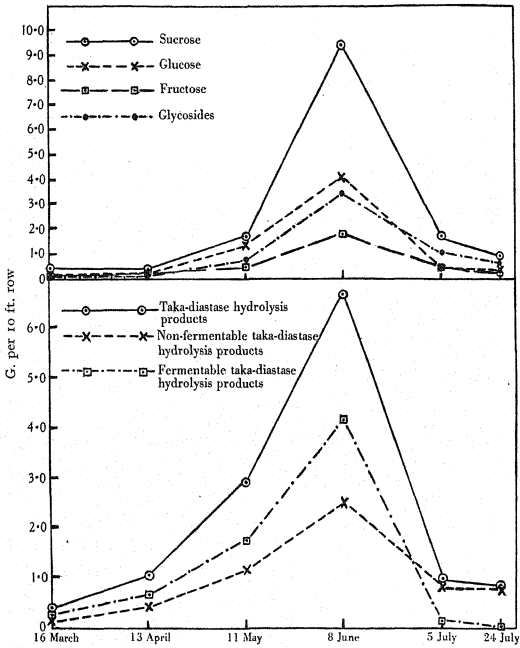

Fig. 12. Progress of carbohydrate accumulation in the leaf-sheaths. The amounts of sucrose, glucose, fructose, and glycoside glucose present in the leaf-sheaths at various times are shown in the top curves. The lower curves show the amounts of taka-diastase hydrolysis products, non-fermentable taka-diastase hydrolysis products and fermentable taka-diastase hydrolysis products present at the same times.

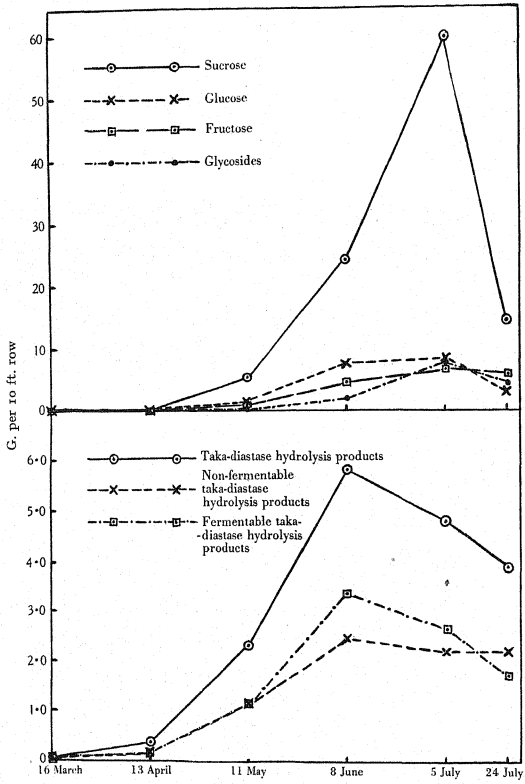

Fig. 13. Progress of carbohydrate accumulation in the stems. The amounts of sucrose, glucose, fructose and glycoside glucose present in the stems at various times are shown in the top curves. The lower curves show the amounts of taka-diastase hydrolysis products, non-fermentable taka-diastase hydrolysis products and fermentable taka-diastase hydrolysis products present at the same times.

reserve carbohydrates estimated as fermentable and non-fermentable taka-diastase hydrolysis products attained their maximum amounts earlier (8 June). The total amounts of all soluble carbohydrates in the ears (Table V) attained their maximum on 5 July, but the amounts of fermentable and non-fermentable taka-diastase hydrolysis products and also the dry weight were greatest on 24 July; presumably continuing to increase during the few succeeding days to harvest.

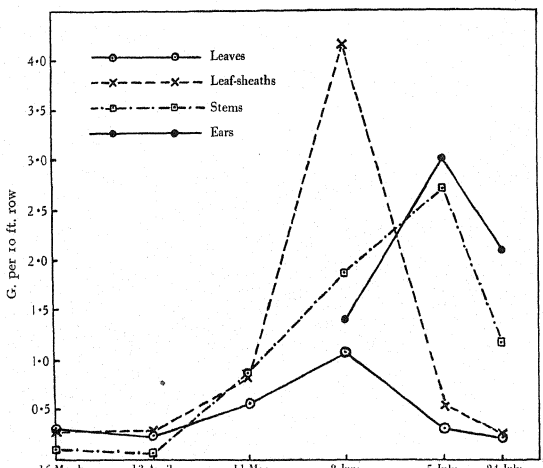

Fig. 14. Progress of fructosan accumulation in the leaves, leaf-sheaths, stems and ears of wheat.

The intervals between sampling dates were too long to permit of detailed discussion of interrelationships of carbohydrates within tissues or between the carbohydrates in the component tissues, but it is clear that the definite metabolic drifts determined as occurring in whole plants were the summations of individual drifts within each plant component. On the whole we can say that the definite drifts followed by the carbohydrates in the leaves passed through their

stages earlier in the season than those of the carbohydrates in the leaf-sheaths, and the metabolic development of the carbohydrates within the leaf-sheaths preceded in time the similar developments within the stem. The carbohydrates of the ears developed similarly and simultaneously with those of the stem except for the taka-diastase hydrolysis products, both fractions of which continued to increase in the ear to the last sampling date when the total dry weight also was highest.

The total amounts of fructosans within each plant component (Fig. 14) are of interest. Definite drifts of the total amounts occurred within each tissue, maximal values being attained in the leaf and leaf-sheath on 8 June and in the stem and ear tissues on 5 July (lower stem on 8 June, Table V) suggesting its function to be that of a temporary reserve carbohydrate. The highest amount in the ear did not coincide with the date of maximum dry weight of ear tissue as the fructosan concentration was falling rapidly between 8 June and 24 July (Fig. 9).

Summary and discussion

The leaves, leaf-sheaths, stems and ears of samples of Rivets wheat collected at sunrise through part of the season 1934–5 each showed well-defined developmental drifts of the percentage amounts of various carbohydrates including fructose-yielding polysaccharides (fructosans).

The drifts within each plant component were similar to each other but had different time relations: thus the percentage of cane-sugar within each tissue rose to a peak value and then fell, but these peak values were attained at different dates in the different tissues: leaf, before 16 March; leaf-sheath, 8 June; stem, 5 July; ear, 5 July. Similar time differences were found between the plant components for each estimated carbohydrate.

The long (monthly) intervals between sampling rendered it difficult to determine precisely the forms of the drifts or to give detailed accounts of the interrelations of carbohydrates within each plant-component or between plant-components. However, certain conclusions may be drawn and suggestions made.

In each plant-component the dry matter percentage rose from March to the end of the season, but the sugars in the leaf tissue fell in percentage amount throughout that period, their rate of utilization during the first month or two, mainly in the formation and differen-

tiation of tissue within the leaf and later translocation to the more rapidly growing leaf-sheath and stem tissues, exceeding their rate of formation within the leaf. In the leaf-sheath sugars fell in percentage amount from 8 June onwards, suggesting that the vigorous growth of the stem during June resulted in a withdrawal to some extent of carbohydrate supplies from the leaf-sheath and leaves. In the stem the sugars (except fructose) fell from 5 July to the end, and the same occurred in the ear. This final fall in all tissues between 5 and 24 July must be ascribed to the low values of the critical concentrations of sugars for starch formation in the ear resulting in the piling up of starch. The relatively high fructose concentration in the stem (also reported for whole plant (Barnell, 1936)) on the final sampling date may have been due to reduced respiration rate of stem tissue at this time, assuming Onslow's hypothesis of γ-fructose being the preferentially respired sugar (Onslow, 1931); or, it may have been due to a higher value of the critical concentration for fructose than for the other sugars, but this is not supported by the presence of a low fructose concentration in the ear at this date. The "bound" or glycosidic glucose increased in the leaf till 8 June to 5 July; leaf-sheath, 8 June; stem, 5 July; ear, 5 July; differing therefore from the sugars in not being drawn upon in any tissue till after 8 June when the vigorous stem growth provided an adequate reason for the decreasing amounts of soluble carbohydrates in all tissues. In each tissue the glycosidic glucose peak value was attained after, or at, the same sampling date as the glucose maximum (their apparent occurrence on the same date in the leaf-sheath and ear was probably due to the infrequent sampling), supporting the suggestion made from data for the whole plant (Barnell, 1936) that the velocity constant for the condensing direction of glucose-glycoside reaction in the plant tissues is greater than that for glycoside hydrolysis.

The absence of true starch in all tissues except ears in these early morning samples was confirmed, but fructosans were present and followed well-defined drifts; particularly noteworthy being the high concentration in the young ear (8 June) and its rapid fall as ear development and starch formation proceeded.

The fermentable (glucose) fraction of the taka-diastase hydrolysis products showed little change in the leaves through the season; little change was shown in the leaf-sheaths till 8 June and then the percentage amount fell to zero on 24 July; in the stem there was none present on 16 March but a fairly constant amount during April and May to 8 June, after which it fell slightly. There was little change in

the non-fermentable (pentose) fraction in the leaves and leaf-sheaths during the season, but in the stem the percentage amount fell from May onwards, finally rising slightly between 5 and 24 July. Both the fermentable and non-fermentable fractions increased during the development of the ears, the former rising to over 20% of the fresh weight on 24 July.

Growth of the whole plants in terms of dry weight per 10 ft. row samples was small between March and April and during this time accumulation of carbohydrates was mainly confined to the leaves, little change taking place in the total amounts per 10 ft. row in leaf-sheaths or stems. Accumulation in these tissues began in April, while in the leaves the amounts of sucrose and fructose fell consistently from April onwards and of glucose from May onwards, the leaf carbohydrates apparently being drawn upon, to some extent, by more vigorously developing parts of the plant. Accumulation of sugars continued in the leaf-sheath till 8 June and then fell, accumulation continuing however, in the most vigorously developing tissue, the stem, until 5 July.

Substances which may be regarded as reserve carbohydrates, the fermentable and non-fermentable fractions of the taka-diastase hydrolysis products, fructosans and glycosides, accumulated in all tissues till 8 June (glycosides and fructosans in the stems till 5 July) and then began to decrease in total amounts. The time from 8 June onwards is a period when rapid elongation and increase in dry matter (mainly cellulose) was proceeding in the stems and of ear development so the demands on carbohydrate supply would be great—too great, apparently for the photosynthetic activity of the assimilating tissues to supply—and so all the estimated carbohydrates decreased in total amount over this period except in the ears where the starch (fermentable taka-diastase hydrolysis products) and pentosans (non-fermentable hydrolysis products) increased.

I have to acknowledge the careful technical assistance given by Mr F. E. Maskell.

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THE VARIABILITY IN, AND NATURE OF THE SPIKELETS COMPOSING THE FASCICLE IN *LAMARCKIA AUREA* MOENCH.

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(With four figures in the text)

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INTRODUCTION

THE inflorescences used in investigations into the self-fertility of the grasses at the Welsh Plant Breeding Station are threshed out individually by hand. This is a somewhat tedious process, but it enables any exceptional characteristics which may appear to be observed.

In the case of *Lamarckia aurea*[1] (Golden Top) each group of spikelets, or fascicle, was expected to yield only one caryopsis, but specimens were encountered which contained two or more (Beddows, 1935). These and the various other irregularities met with were recorded, and the data so obtained form the basis of this paper.

The original seeds were obtained respectively from Algeria, Czechoslovakia, Bulgaria and America.

[1] Synonyms which have been used for *Lamarcki aaurea* Moench. are: *Cynosurus aureus* L., *Chrysurus cynosuroides* Pers., *Chrysurus aureus* P. Beauv., *Pterium elegans* Desr., *Tinaea elegans* Garz., and *Achyrodes aureum* Kuntze.

The plants investigated were grown singly in pots or spaced in boxes, and grown to maturity in a large cool greenhouse.

THE VARIABILITY OF THE UNITS COMPOSING THE FASCICLE

(*a*) *Variation in number and type of spikelet*

Most of the authors consulted[1] described the fascicle as consisting of four spikelets, of which three are sterile and one is fertile. The sterile spikelets are said to be made up of a number of alternate overlapping, imperfect florets, each represented by its inferior palea only. The fertile spikelet is described as terminal with two, or rarely three, florets. The figures given by Reichenbach (1834) and Phillips (1931), however, seem to include an additional structure, probably the second fertile spikelet, although no reference to this is made in the respective texts. Schlechtendal *et al.* (1881) actually give the number of spikelets as five, but they do not classify them.

Varying numbers of spikelets have also been reported, ranging from a fascicle of one fertile and two sterile spikelets (Nees ab Esenbeck, 1843), to a fascicle of five sterile and three fertile spikelets (Camus, 1935).

Arber (1928) confirmed Beauvois (1812) as to the presence of a second hermaphrodite spikelet attached to the lowest of the sterile spikelets. As a result of an examination of living and herbarium material she came to the conclusion that the two forms of fertile spikelet might be a characteristic feature of the species. She called the less obvious fertile form a "reduced-fertile" spikelet, to distinguish it from the more readily observed "principal-fertile" spikelet.

Hitchcock (1935) amended his earlier description of the fascicle to include this reduced fertile spikelet.

In the course of the present work it was found that this reduced fertile spikelet was absent in only 0·55% of the 8549 fascicles examined. It is evident, therefore, that the presence of this structure in the fascicle is very much more characteristic than its absence.

It is also probably significant that where the fascicle consisted of five spikelets (Table I, group *h*) these were invariably of the type

[1] Linnaeus (1753), Lamarck & Candolle (1805), Reichenbach (1834), Nees ab Esenbeck (1843), Grenier & Godron (1856), Cesati *et al.* (1867), Bentham & Hooker (1883), Hackel (1887), Baillon (1893), Fiori & Paoletti (1896), Husnot (1896), Lamson-Scribner (1900), Ascherson & Graebner (1901), Battandier & Trabut (1904), Hitchcock (1920), Bews (1929), Ewart (1930), and Phillips (1931).

three sterile, one principal fertile and one reduced fertile. That is to say, in no case was any spikelet in the common five spikelet fascicle replaced by one of a different type. These standard fascicles accounted for the great majority (95–99 %) of the 7618 fascicles investigated from this point of view.

TABLE I. *Showing the number of spikelets per fascicle, the distribution of the spikelet types within the different groups, and the number of times each group occurred in the fascicles examined.* Lamarckia aurea *grown at Aberystwyth,* 1935 *and* 1936

		Spikelet types within the fascicle			Number of each group found			
Spikelets per fascicle	Group	S (sterile)	R.F. (reduced-fertile)	P.F. (principal-fertile)	Czecho-slovakian	Algerian	U.S.A.	Bulgarian
2	*a*	1	0	1	—	2	—	—
	b	1	1	1	—	1	—	—
	c	2	0	1	3	26	6	—
	d	2	1	0	—	3	—	—
3	*c* or *d*	(i.e. classification uncertain)			2	10	—	—
	e	2	1	1	13	90	4	—
4	*f*	3	0	1	—	2	—	—
	g	3	1	0	8	13	—	—
5	*h*	3	1	1	1012	3050	814	2725
6	*i*	3	1	2	—	1	—	—
	j	4	1	1	—	9	—	—
7	*k*	5	1	1	—	1	—	—
8	*l*	5	1	2	1	—	—	—
9	*m*	6	1	2	—	—	—	1
10	*n*	6	2	2	—	1	—	—
			Fascicle totals		1039	3209	824	2726

Where the fascicles consisted of less than five spikelets, a spikelet of any one of the types might be missing. There were 171 fascicles with from two to four spikelets. Of these the sterile spikelets gave only 151 or 29·4 % of their normal complement of 513, while the reduced-fertiles had thirty-nine or 22·8 % and the principal-fertiles twenty-four or 14·0 % of their normal number (171). It therefore appears that the principal-fertile spikelet is least liable to be omitted and one of the sterile spikelets the most liable.

When the types with more than the normal five spikelets are examined, we find that there is a total of only fourteen for consideration. In these the sterile spikelets exceeded the normal number by nineteen or 45·2 %, the principal-fertile by 28·5 %, and the reduced-fertile by 7·1 %.

Taking all the groups together the most common exceptions were

(*e*) and (*c*), the former with only one sterile spikelet missing, and the latter with the reduced-fertile and a sterile spikelet missing.

It would thus appear that the least stable type of spikelet, in so far as number per fascicle is concerned, is the sterile rather than the reduced-fertile type.

An examination of the data in their relation to the origin of the seed reveals the fact that the Algerian with 3209 fascicles gave all the fascicle types except those of eight and nine spikelets each. The Californian sample gave only two types besides the normal, and both had less than five spikelets. In this case, however, only 824 fascicles were examined. The Bulgarian material with 2726 fascicles examined gave only one exceptional fascicle and this had nine spikelets, but the original sample of seed, as received from Sofia, actually contained five fascicles each with less than five spikelets.

With regard to the position of the more elaborated fascicles in the inflorescence, it was observed that they usually occurred in the lower part, but the rule was by no means absolute, specimen No. 41 (Fig. 1), for instance, was developed in the upper part of a panicle.

If the standard fascicle be regarded as consisting of five spikelets, with the reduced-fertile arising from the pedicel of the lowest sterile spikelet, and the principal-fertile as terminal or branching from the pedicel of the uppermost sterile spikelet, it will be seen that the additional spikelets may arise in various positions. Thus, each of the fascicles Nos. 36–40 (Fig. 1) possess an extra sterile spikelet. In Nos. 36 and 37 it is borne on a branch from the pedicel of the lowest sterile spikelet, in No. 38 on that of the second, and in No. 39 on the third. In No. 40 the two additional sterile spikelets are attached respectively to the lowest and second sterile spikelets.

(*b*) *The fertility of the different spikelets as indicated by caryopsis formation*

It was at one time thought that the fascicle produced only one caryopsis. Arber (1934), however, found that in thirty-seven fascicles out of ninety-four examined a caryopsis had also been developed in the reduced-fertile spikelet.

A detailed study of the material grown at Aberystwyth has shown that this two-seeded condition of the fascicle is fairly common, and was found in plants from each of the four samples of seed used. Further, it showed that although the additional caryopsis most frequently occurs in the reduced-fertile spikelet, it may occur in any spikelet. From 8248 fascicles examined 13,243 caryopses were

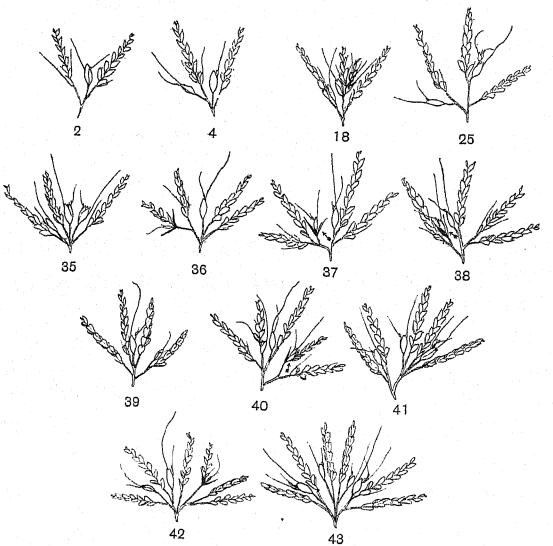

Fig. 1. Some examples of the variation in spikelet type to be found in *L. aurea*. The specimens carry the same numbers in the text-figures as they bear in Fig. 4. The glumes have been omitted from most spikelets in order to make the drawings clearer. (2) S2+S3+P.F. S2 has two caryopses, note awns. (4) S3 missing. P.F., R.F. and S1 each have a functional floret. (18) The second floret of the nine-flowered P.F. spikelet has given rise to a secondary spikelet with four florets. (25) A normal 3S+P.F.+R.F. spikelet. (35) A second P.F. spikelet has arisen here as a branch of the S2 spikelet. (36) A sterile spikelet of seven florets is subtended by the same glumes as the R.F. spikelet. (37) S1 has branched to give a secondary S1 (*b*) spikelet. The inferior glume of the R.F. spikelet has been transformed into an awned floret—marked by arrow. (38) S2 has produced a secondary S2 (*b*) spikelet. N.B. awn-like structure in position of R.F. glume. (39) Shows second S3 spikelet. (40) Five S-type spikelets, S1 (*b*) with nine florets replaces the inferior glume of the R.F. spikelet. S2 (*b*) also with nine florets has a caryopsis in the lowest floret. (41) 5S+3F. spikelets—the S1 branch has been replaced by a normal fascicle with functional R.F. and P.F. spikelets. (42) 6S+2P.F.+1R.F. spikelets, the single-flowered R.F. spikelet and the eight-flowered S spikelet have both arisen within the same pair of glumes; compare similar condition in specimen (36). (43) 6S+2P.F.+2R.F. spikelets. This fascicle is composed of two almost identical normal fascicles. The S3 and P.F. spikelets to the right arise from practically the same position on the pedicel; there were, however, four glumes present. All ×1·5, and somewhat diagrammatically represented.

obtained, giving an average of 1·60 per fascicle. Of these 2377 (or 17·94%) were more or less poorly developed, while the remaining seeds were fully developed.

A more detailed examination of the figures in Table II shows that out of the total of 13,243 caryopses obtained, 642 (approximately 5%) were produced by the sterile spikelets, and of these about 80% were normally developed. A higher proportion of the total caryopses was produced by the reduced-fertile spikelets (about 33%), but nearly half of these were poorly developed. The principal-fertile spikelets produced 62% of the total caryopses, and of these rather less than 3% were poorly developed.

The Algerian lot with 2617 fascicles examined gave the highest yield of well-developed caryopses per 100 spikelets (174·3), but it is somewhat doubtful whether any significance can be attached to the variation in this respect between the different lots.

TABLE II. *Showing the distribution and number of caryopses (light-heavy and heavy seeds*) among the different spikelet types found within the fascicles of* L. aurea. *Data collected from forty-four panicles from thirty-nine plants spaced in boxes, greenhouse. Aberystwyth* 1935 *and* 1936

Country of origin	Fascicles examined	Caryopsis type	Number of caryopses found in the: Sterile spikelets	Reduced-fertile spikelets	Principal-fertile spikelets	Totals	Heavy seed per 100 spikelets
Czecho-slovakia	1624	Light-heavy	4	297	9	310	—
		Heavy	2	184	1567	1753	107·3
U.S.A.	1282	Light-heavy	23	476	129	628	—
		Heavy	0	456	1152	1608	125·4
Algeria	2617	Light-heavy	104	348	35	487	—
		Heavy	509	1441	2612	4562	174·3
Bulgaria	2725	Light-heavy	0	897	55	952	—
		Heavy	0	326	2617	2943	108·0
Totals	8248	Light-heavy	131	2018	228	2377	—
		Heavy	511	2407	7948	10866	131·7
		Total caryopses	642	4425	8176	13243	—

* The light-heavy caryopses were flat and brown; the heavy seeds were well developed, plump and opaque.

The presence of caryopses in the so-called "sterile" spikelets does not appear to have been previously recorded. These functional sterile spikelets which were far more frequent in the plants from the Algerian sample than in the Czechoslovakian or American were not once recorded in the Bulgarian fascicles examined.

The caryopsis is usually developed in the basal floret, but the second floret may also be perfect and functional not only in the principal-fertile spikelet[1] but also in the sterile spikelets. No reduced-fertile spikelet, on the other hand, ever gave more than one caryopsis. Viable seed was obtained from all three types of spikelet and the resulting plants, if derived from seed from the same parent, produced fascicles giving a very similar range of variations.

It is, therefore, evident that the names "sterile" and "fertile" as applied to the spikelets of *L. aurea* are descriptive of relative rather than absolute fertility values.

(c) *The number of florets in the various spikelets*

(i) *The principal-fertile spikelet.*

The (principal) fertile spikelet was usually regarded as having two florets, yet Linnaeus (1753), Nees ab Esenbeck (1843), Petermann (1849), and an unknown writer in the *Cottage Gardener* (1857) either described or illustrated a third (rudimentary) floret.

The material studied at Aberystwyth has shown that while the two-flowered condition was the more usual—68 % of the 7601 spikelets examined—the three-flowered spikelet was also fairly common (28·5 %). It was further discovered that the number of florets which can be developed in these principal-fertile spikelets may actually range from one to as high as thirteen (see Fig. 2). Of the spikelets with these high numbers, those most frequently found had four, five and six florets, but even when considered as one group they only accounted for a relatively small percentage of the total spikelets investigated. Thus for the Algerian material, the four to six flowered spikelets accounted for 11·7 % of the total of 1012 principal-fertile spikelets, the Czechoslovakian 3·0 % of 3050, and the Californian 1·3 % of 814 spikelets.

The widest range in flower number was provided by the Algerian, but that given by the Czechoslovakian followed it very closely (see Table III). The Californian material did not produce any principal-fertile spikelets with more than six florets, while in that from Bulgaria the spikelets were almost entirely two-flowered (99·3 %), the only other numbers obtained being one and three, each represented by four examples.

[1] Phillips (1931) describing the (principal) fertile spikelet states: "Valves 2 or sometimes valve 1,... each subtending a pale and a bisexual flower, or the upper valve empty or subtending a male flower."

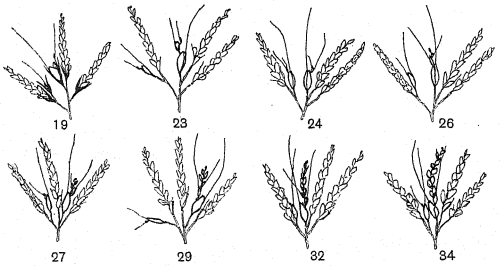

Fig. 2. A few types of multiflowered P.F. spikelets in *L. aurea*. The glumes have been omitted except in specimen (19). The P.F. and R.F. spikelets have been more heavily lined than the S spikelets. (19) P.F. with two florets, R.F. one. (23) P.F. with three florets, R.F. two. (24) P.F. and R.F. both with three florets. Lowest floret in each contains a caryopsis. (26) P.F. four florets; R.F. two. (27) P.F. five florets. (29) P.F. seven florets. (32) P.F. ten florets. (34) P.F. thirteen florets. All ×1·5. Somewhat diagrammatic.

TABLE III. *Showing the number of florets found in the reduced-fertile and principal-fertile spikelets, the numbers in combination within the fascicles, and their distribution in each origin lot.* Lamarckia aurea. *Aberystwyth grown plants,* 1935 *and* 1936. *The data were collected from fascicles of type* 3*S*+1 *R.F.*+1 *P.F. only*

Florets in reduced-fertile spikelet	Florets in complementary principal-fertile	Distribution of combinations in each origin lot			
		Czechoslovakian	Algerian	Californian	Bulgarian
1	1	—	—	—	3
1	2	—	33	—	267
1	3	—	3	—	—
2	1	—	—	—	1
2	2	520	1117	765	2450
2	3	326	1776	38	4
2	4	48	36	3	—
2	5	29	35	3	—
2	6	31	22	5	—
2	7	15	9	—	—
2	8	6	6	—	—
2	9	3	6	—	—
2	10	1	3	—	—
2	11	—	1	—	—
2	12	—	—	—	—
2	13	—	1	—	—
3	3	22	2	—	—
3	4	7	—	—	—
3	5	3	—	—	—
3	6	1	—	—	—
	Total fascicles	1012	3050	814	2725

The absence of a twelve-flowered spikelet from the standard (3S+1 R.F.+1 P.F.) fascicles examined was probably due to chance alone. A principal-fertile spikelet with this number of florets was, however, found among fascicles with only two sterile spikelets (see Fig. 4, specimens 16 (central spikelet) and 17 (right-hand spikelet)). It may perhaps be argued that the multi-flowered spikelets in these specimens, as well as in Nos. 14 and 15 with ten and eleven florets respectively, are in reality functional sterile spikelets in fascicles without their principal-fertile spikelets. This is, of course, a possible interpretation, and it seems to receive support from the fact that only two definite awns are produced in these spikelets. This difficulty in interpretation rarely arises in the standard fascicles, for then the positions of the pedicels help to fix the identity of the spikelets. Thus although the thirteen-flowered principal-fertile spikelet in specimen 34 (Figs. 2 and 4) has only the two lowest florets awned, it must in virtue of its position be regarded as definitely a principal-fertile spikelet.

(ii) *The reduced-fertile spikelet.*

The number of florets in the reduced-fertile spikelets from the material grown at Aberystwyth showed the very limited range of from one to three.[1] The two-flowered spikelets accounted for 95·5% of the total, and the one- and three-flowered spikelets but 0·5 and 4·0% respectively.

If we consider the flower number relationship of the two fertile spikelets within the fascicle it will be found (Table III) that the most frequent combinations are those of a two-flowered reduced-fertile spikelet with a principal-fertile spikelet of either two florets (63·8%), or three florets (28·2%).

In both fertile spikelets the floret in a one-flowered spikelet was very often reduced to a small awn with a more or less swollen base, the remains of what would normally be the inferior palea. A similar condition is also frequently found in the apical florets of these spikelets. Complete suppression of the florets may also occur (see Fig. 3).

[1] A sample of *L. aurea* received from the Botanical Garden, Brussels, in March 1937 contained six reduced-fertile spikelets with four florets and two with five florets, all relatively well developed. The principal-fertile spikelets present showed a progressive range of from two to ten in the number of their florets. In the sterile spikelets, one of which was functional, the maximum number of florets found was seventeen.

(iii) *The sterile spikelets.*

The number of florets in the sterile spikelets was said to vary between six and ten, but counts by the present writer on twenty random fascicles from each of eighteen panicles representing seventeen plants gave a range of from six to fifteen for the Algerian material and nine to fourteen for the Californian. The figures for the three sterile spikelets within a fascicle showed but slight differences, the averages for 280 (Algerian) S1, S2, and S3 spikelets were 11·4, 11·7 and 11·6 respectively, while for eighty (Californian) fascicles, the comparable figures were 10·9, 11·4 and 11·3. The sterile spikelets may occasionally be reduced to one or two inferior paleae or even to their glumes (see Fig. 3).

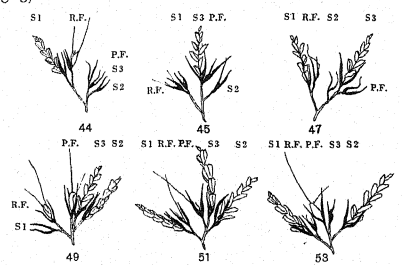

Fig. 3. Examples of spikelet reduction in *L. aurea*. All the spikelets have their glumes represented in solid black. (44) S2 and P.F. apparently reduced to their glumes. S3 shows a rudimentary floret. (45) S2 and P.F. each appear to have one minute floret, while S1 has two. (47) S2 and P.F. reduced to their glumes, R.F. has one very small floret with long delicate awn. (49) S1 consists of glumes only. (51) P.F. has two florets but these very small so that it closely resembles the R.F. spikelet in character. (53) S3 has two small deformed florets. All × 1·5. Somewhat diagrammatic.

THE NERVES AND AWNS OF THE INFERIOR PALEAE

Very little information is available in the literature regarding the number of nerves to be found in the inferior paleae of the fertile spikelets. The number more often than not is not mentioned at all or is said to vary between one and five. With regard to the sterile spikelets the only reference is that of Nees ab Esenbeck (1843) who gives the number of nerves as constantly less than three.

The present writer examined the paleae of the various spikelets macroscopically. He found that the inferior palea of the basal floret

in the principal fertile spikelet had five nerves including the keel. This number was also found in the comparable floret of the reduced-fertile spikelet, but only when it is well developed. In the sterile spikelets the inferior paleae are ordinarily three nerved, except when one of them forms part of a perfect floret when the number is five. The second and succeeding florets (= inferior paleae) in sterile as well as fertile spikelets seem to be three nerved.

The awns in the fertile spikelets are described as arising either from between the bifid apex or from just below the apex of the inferior paleae. In the case of the sterile spikelets even the possibility of awns being present is not mentioned, although Beauvois (1812, Pl. XXII, fig. V) shows a definite awn on the lowest floret of one of the sterile spikelets.

The varying descriptions given for the awn in the fertile spikelets undoubtedly refer to different aspects of the same condition for the awn is an extension of the central nerve. The derivation of the awn is the same whatever the spikelet type in which it may be developed.

Discussion

The principal-fertile and sterile spikelets, although ordinarily quite readily distinguishable, may, however, approximate to each other not only as regards number and fertility of their florets, but also in respect of nervation and awnedness. The similarity is in certain cases so close that the true nature of such spikelets is chiefly to be recognized by the relative positions of their pedicels within the fascicles.

Fascicles with spikelets differing in number or arrangement from that found in the standard form accounted for less than 10% of the total fascicles examined. In the case of the spikelets themselves, however, even those in standard fascicles, the range of forms was very wide, and involved not only the number of florets, but their degree of development and function as well. The variations found, especially in the case of the fertile spikelets, might be so arranged as to give a continuous series of forms, ranging from spikelets of greater complexity than the standard, to those showing almost complete suppression of the florets.

The general trend of the variations is probably in the direction of greater simplicity, but the different spikelets are not equally affected. Thus in the principal-fertile spikelet the number of florets diminishes, but in the reduced-fertile spikelet decrease in number of florets is also accompanied by considerable loss of function in the basal floret. In the sterile spikelets, on the other hand, loss of function is practically

complete and the awns are largely suppressed, but there is relatively little reduction in the number of florets. Loss of function, it is true, has occurred in the principal-fertile spikelet also, and in fact is almost complete in the second floret, but in the basal floret it is exceptional.

Arber (1928, p. 181; 1934, p. 184) as a result of her detailed studies into the development of the florets in numerous grasses, including *L. aurea*, considers that "the Gramineae...suffer from a definite inherent trend towards some degree of sterilization of the reproductive shoot". She therefore regards the two fertile spikelets found in *L. aurea* to be similar and explains the observed differences as due to the process of sterilization, having proceeded further in the lower (or reduced) fertile spikelet than in the upper (or principal) fertile spikelet.

Whatever the factor or factors which control the appearance of the various spikelet types found in this species, the data now being accumulated at Aberystwyth not only confirm the close relationship of the two fertile spikelets but also suggest that *all the spikelets in the fascicle (sterile as well as fertile) are fundamentally the same.*

It is interesting to note that although reduction of the florets in a spikelet may be almost complete, the two glumes remain practically unaffected (compare specimens 44–53 in Fig. 4, and those in Fig. 3).

Legend to fig. 4.

Fig. 4. (1) A fascicle of *L. aurea* of only three spikelets, one sterile (S3), and two principal-fertile spikelets. (2)–(18) Fascicles with two sterile spikelets, and one or two fertile spikelets. Specimens 4–17 show in their principal-fertile spikelets an increase in number of florets of from two to twelve. In specimen 13, with a principal-fertile spikelet of nine florets, the four lowest are awned, but in 14, with ten florets, 15 with eleven florets, and 16 and 17, each with twelve florets, the two lowest only are awned. On this account, and in the absence of the third sterile spikelet (S3) the classification of these multi-flowered spikelets is somewhat uncertain so that the fascicles may be regarded as consisting not of the type 2S+R.F.+P.F., but rather of the type 3S+R.F. (19)–(34) These fascicles, which are all of the type 3S+R.F.+P.F., show in their principal-fertile spikelets a range of from two to thirteen florets. In specimens 26–33 the number increases progressively from four to eleven, while in 34 the number is thirteen. In specimens 22 and 23 each P.F. spikelet has developed two caryopses. In specimen 19 the reduced-fertile spikelet has only one floret. (35) Contains an additional P.F. spikelet, borne on sterile spikelet S2, which is very similar to that found in the normal position on S3. (36) Contains an extra spikelet of seven florets subtended by the same pair of glumes as the reduced-fertile spikelet. (37)–(39) These fascicles have four sterile spikelets, of which the additional member is borne respectively on sterile spikelets S1, S2 and S3. (40)–(43) These fascicles with eight to ten spikelets were the most complex types found—see Fig. 1 for full description. (44)–(53) These specimens illustrate the degree of reduction which can occur in the various spikelets of the fascicle—see Fig. 3 for description. All ×0·8.

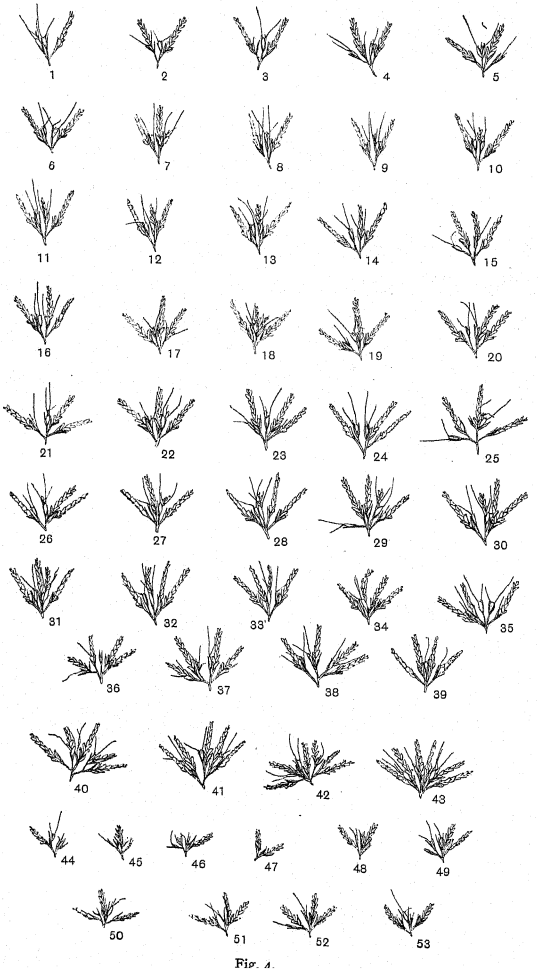
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Fig. 4.

It occasionally happens, however, that a glume is replaced by an entirely different structure, as for example in the reduced-fertile spikelets of specimens 38, 37 and 40. In these a glume has been replaced respectively by an awn, a spikelet with at least one floret which is quite well awned, and by a nine-flowered spikelet (see Fig. 1).

The influence which the place of origin of the seed samples may seem to have had on the types produced was probably accidental and due to conditions of sampling. The samples may quite conceivably have been gathered from single plants or from but few plants in a very restricted area. In such cases, especially with a normally self-fertilizing species like *L. aurea*, only some of the types possible for the species as a whole would be obtained in the progeny. It may be necessary to study a large number of plants from different "wild" habitats before the relationships of the possible and perhaps of even the present divergencies in fascicle type can be adequately assessed.

The degree of development reached by the reduced-fertile spikelet is considered by Arber (1928) to depend probably on the vigour of the panicle. That factors other than vigour are also concerned in this is shown by the fact that it was a common experience at Aberystwyth to find different grades of reduced-fertile spikelets within the same panicle. The stages found might range from three-flowered spikelets with a well-developed caryopsis in the basal floret to spikelets with but one floret and this more or less aborted. The principal-fertile spikelets also showed a comparable range in the number and development of the florets, but the reduction is not so extreme. Thus in eleven sister plants (the parent was protected against the possibility of cross-fertilization) the minimum range in flower number was from two to five and the maximum range two to ten.

In the case of the cross-fertilized species *Lolium perenne* it has been demonstrated by Jenkin (1931*a*) that the number of florets found in the spikelet is influenced by vigour. He points out, however, that the range of variation possible under uniform conditions is limited by the genetical make-up of the individual plant concerned.

The qualitative and quantitative changes found in the spikelets of *Lamarckia aurea* are in several respects comparable to those described for *Arrhenatherum* Beauv. by Jenkin (1931*b*). In tall oat grass the spikelet is two-flowered, the lower being male and the upper hermaphrodite. Jenkin considers that it is rare to find, in nature, plants with spikelets containing more than two florets, or with their basal floret female fertile. He has, however, by breeding, been able

to produce plants with spikelets normally three-flowered, as well as occasional plants with the fourth floret in the spikelet well developed. He found that the lowest floret could also be fully female fertile, and that in certain plants such florets gave, upon selfing, as many caryopses as did the normally perfect second floret.

Some of the synonyms under which *Lamarckia aurea* has been known show that its systematic position in relation to *Cynosurus* was at one time uncertain, but they are now regarded as in quite distinct genera.

The sterile spikelet in *C. cristatus* has also been recorded as fertile, but it is of very rare occurrence (Hegi, 1906). Thoenes (1929) records two instances of a three and a four flowered spikelet apparently quite normal in structure being found in the axil of a glume of a sterile spikelet. The fertile and sterile spikelets in *C. cristatus* now seem to have little in common, and it is probable that they diverged from a possible common spikelet type much earlier in phylogeny than has been the case in the spikelets of *Lamarckia aurea*.

Summary

1. The descriptions of the fascicles of *Lamarckia aurea* Moench. given by various authors are summarized and compared.

2. The fascicle in its common (or standard) form is to be regarded as consisting of five spikelets which are classified into one principal-fertile, one reduced-fertile, and three sterile spikelets. The principal-fertile spikelet has two or three florets, the lowest of these being usually perfect and functional. The reduced-fertile spikelet has two florets of which the basal is not infrequently functional. Each of the three sterile spikelets contains from six to fifteen florets, all of which are imperfect and neuter.

3. All spikelet types may show quantitative and/or qualitative variations. Thus the principal-fertile spikelet may contain up to thirteen florets, and the second as well as the basal floret may be functional. In the reduced-fertile spikelet the highest number of florets recorded so far is five. In the sterile spikelets the two lowest florets may very occasionally become perfect and functional.

4. The most complex fascicle examined contained ten spikelets; six sterile, two principal-fertile, and two reduced-fertile spikelets.

5. Each or all of the five spikelets in the fascicle may be functional, but the maximum number of caryopses found in a fascicle was six, and in a spikelet two.

6. The nervation in functional and non-functional florets of sterile spikelets is similar to that in comparable florets of fertile spikelets.

7. The position and derivation of the awn, whether in functional or non-functional florets, is apparently the same for all the spikelets in the fascicle.

8. It is suggested that all the spikelets in the fascicle are homologous.

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TAXONOMY AND RELATIONSHIP IN THE GERANIALES IN THE LIGHT OF THEIR CYTOLOGY

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(With 68 figures in the text)

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PART I

Introduction

In recent years various successful attempts have been made to elucidate taxonomic relationships by means of investigations into cytology.[1] These have in the main been restricted in scope to a single genus (e.g. *Primula* (Bruun, 1932), *Viola* (Clausen, 1927, 1929)) or to the limits of a single natural family (e.g. Cruciferae (Manton, 1932)) consisting of plants with regard to whose general phylogenetic relationship there can be no doubt, and have been confined to elucidating the relationships within such groups. The object of the

[1] Throughout this paper the term cytology has been used in its modern restricted sense, for which karyology is perhaps the correct term.

present paper is to carry out this process as far as possible for the Geraniaceae and to extend it to the relationships of the Geraniaceae with other families of the order Geraniales. The Geraniales were chosen as suitable for this purpose because of the differing positions given by various authors to the families included in the group, especially Limnanthaceae and Balsaminaceae. Hutchinson's (1926) classification has been taken as a basis, as the number of families included by him in the Geraniales is conveniently small.

Objection has been made to the application of cytology in attempts to elucidate the relationships of such comparatively large groups.

An attempt is made below to show that cytological characters in their range of variation, and type of variation within groups of different taxonomic value are analogous to ordinary morphological characters. There seems therefore no valid reason why their use as criteria of relationship should not be extended to groups of comparatively remote relationship, provided it is recognized that resemblances may be due to convergence in just the same way as similar resemblances in morphological characters. It is in this connexion particularly important to recognize that there are few available cytological characters and that it is not possible or desirable to base any classification on cytological characters alone. Such care would seem to be particularly necessary in the case of more remotely allied groups, as it is in such that relationship becomes more uncertain on morphological grounds, since speculations on relationship vary according to which character is considered the most fundamental.

It is proposed in what follows to treat each family and genus separately, and to lead from this to a consideration of the relationships between the genera and families included in the order.

Material and methods

In order to provide the widest possible range of cytological characters in which variation might occur, both mitotic and meiotic divisions were studied.

Various fixatives were tried. For root tips the chrom-acetic-formalin fixative as used by Manton (1932) was found satisfactory. Sections were cut at 10 μ. and stained either by Newton's gentian violet method or by Clausen's (1926) modification of it.

For meiotic divisions several methods were used. For the larger buds (some species of *Geranium, Pelargonium, Impatiens, Tropaeolum, Limnanthes, Linum* and the Zygophyllaceae) the anthers were fixed separately in the fixative just named, or in La Cour's (1931) 2 BE.

For the smaller buds (the remaining species of *Geranium*, *Erodium* and *Oxalis*), buds were fixed whole in the same fixative (chrom-acetic-formalin) after preliminary treatment in Carnoy's fluid for 2 or 3 min., or Carnoy's fluid alone was used for about half an hour. In all instances sections were cut at 10 μ. For staining, Newton's method was used without modification, Clausen's modification being found to leave stain in the cytoplasm.

Haematoxylin staining was tried, but proved quite useless for the Geraniaceae and no improvement on gentian violet for the other families.

Smear preparations were also quite useless for the Geraniaceae, but were of use in *Limnanthes* and the Zygophyllaceae. These were fixed in a chrom-acetic solution and stained by Newton's method.

Considerable difficulty was encountered in making satisfactory preparations of the flower buds of some species owing to the tendency for the cytoplasm to retain stain. This was particularly marked in the Geraniaceae, especially when the buds had to be fixed whole owing to the small size of the anthers. The use of Carnoy's fluid alone prevents this to some extent but is liable to cause shrinkage.

The material has mostly proved unsuitable for a detailed study of meiosis owing to the small number of pollen mother cells in the anther, and the small size of the chromosomes: nothing has been attempted on these lines. All drawings in this paper are ×2600.

Care has been taken to get the plants accurately named, the author being responsible for all identifications. The monographs in the *Pflanzenreich* and the Cambridge herbarium have been the chief sources used for identification.

The plants used have been supplied by various botanic and private gardens. It has not been thought necessary to state either the origin of the material in such instances nor the names under which they were supplied. For a good many species wild material has been available; for these the locality is given. The writer is indebted to several correspondents for such material.

The writer's thanks are also due to Miss E. R. Saunders, who suggested the problem, and to Prof. F. T. Brooks, F.R.S.

THE VALUE OF CYTOLOGICAL CHARACTERISTICS AS CRITERIA OF RELATIONSHIP

The value of cytological criteria for distinguishing between species has been recognized for some years. The present section attempts to show that these criteria may also be applied to distinguish higher

taxonomic groups, especially when considered in relation to morphological criteria, in spite of the great variability in each group of criteria considered separately, even within small groups of species or within the same species. Darlington (1937) shows that the characteristics of chromosomes can be divided into two classes, those that are genotypically controlled, and those that are inherited directly. The latter class consists of (1) chromosome number and (2) chromosome morphology. Of the former class two characteristics have been used to some extent in this paper: (3) size of chromosomes, considered as a complement (as opposed to size relative to each other, which falls into class (2) above), and (4) frequency and distribution of chiasmata.

A fifth characteristic, often giving valuable information, is the behaviour of chromosomes at meiosis in respect to such phenomena as secondary association and irregular divisions. This is a direct function of the genetic relationship of the chromosomes to each other.

Considering these criteria separately:

(1) *Chromosome number*

It has for some time been appreciated that chromosome number may afford a satisfactory method of distinguishing between species (e.g. *Viola arvensis* $n=13$, *V. tricolor* $n=17$ (Clausen, 1929). Difference in number is not, however, an invariable criterion; for example, in *Silene ciliata*, Blackburn (1928) found strains with $n=12$, 24 and 96 which differed in no morphological character. In such instances the numbers nearly always form a polyploid series and sterility between the strains is usual.

Instances may also be quoted where chromosome number serves to distinguish between genera, thus Chiarugi (1925) found in the Cistaceae that *Cistus* and *Halimium* have the basic number 9, and *Helianthemum* the basic number 8. Most systematists have included *Halimium* in *Helianthemum* rather than in *Cistus*, or at least regarded the two former genera as more nearly related to each other than to the latter, but Stapf (1928) has recently disagreed with this on purely morphological grounds, regarding *Halimium* as more closely related to *Cistus*. Genetical evidence points in the same direction, hybrids having been recorded between *Cistus* and *Halimium* (Warburg, 1930) but not between *Halimium* and *Helianthemum*. In this example, therefore, chromosome number confirms a relationship suspected on other grounds.

There also exist genera and larger groups in which the chromosome number is constant throughout (e.g. gymnosperms), and perhaps commonest of all, genera in which polyploid series occur, though the basic number remains the same (e.g. *Rosa*, *Triticum*, *Chrysanthemum*, where the basic number is 7, and the subfamily Pomoideae of the Rosaceae, where it is 17). Such instances can be multiplied indefinitely.

(2) *Chromosome morphology*

This is interrelated with chromosome number; thus in *Fritillaria* (Darlington, 1937) the variation of number in different species has been shown to be due to the presence of two short chromosomes in some species as compared with one long one in others. The presence of satellites also is often useful as showing the possible phylogeny of various forms. In the plants considered in this paper no striking variations in morphology occur either within the species or between the groups.

(3) *Size of chromosome complement*

Here great variation sometimes occurs within a group (e.g. the tribe Tradescantiae). On the other hand, the size may be constant throughout a comparatively large group.

(4) *Distribution and frequency of chiasmata*

This is considered here as an example of a class of phenomena concerned with chromosome behaviour which it may be possible to use as indications of affinity. Darlington (1937, pp. 110–11) gives a table classifying organisms according to the behaviour of chiasmata at metaphase. In general this appears to be constant within the genus, an exception being *Fritillaria*, where the species are divided between two of the classes.

Darlington's classes are:

(1) Localized proximally: slight movement.
(2) Distributed: slight movement.
(3) Equilibrated: incomplete terminalization.
(4) Fused: complete terminalization.

The classes are according to the distribution of chiasmata at the first metaphase of the largest chromosomes of the organism.

(5) *Chromosome behaviour at meiosis*

The differences here are mainly important as indicating that hybridization or reduplication has occurred. Secondary association of chromosomes may persist for a long time in the history of a group (e.g. the Pomoideae) and is therefore often of value.

The above facts have been set out here in order to have them assembled in convenient form so as to afford a basis of comparison with ordinary morphological characters, for which reason also those relating to chromosome number have been set out in a more extended form.

If any ordinary morphological criterion is considered, it will be found to vary in value from group to group in a similar way to that of any of the cytological criteria set out above. As an example, the form of and number of carpels in the gynaecium may be taken. In some families (e.g. Leguminosae, Labiatae, Cruciferae) both the number of carpels and their arrangement is constant throughout the family. At the opposite end of the scale are such families as the Rosaceae, where both the carpels and the number of ovules in each carpel vary from one to many. The carpels of this family may be free or fused and the fruit drupaceous, pomaceous, follicular or achenial. Here, then, in the value of the characters of the gynaecium in classification there is a variation parallel with that found in different groups with respect to chromosome number.

The same applies to characters used to distinguish genera or species.

Similarly, if attempts are made to classify plants on the basis of a single character, the groups arrived at contain a heterogeneous assemblage of unrelated plants. Linnaeus, for example, classified plants first on the number of stamens, and secondly on the number of carpels, but such characters are not always constant within the species. Thus, though in general two carpels are constant throughout Solanaceae, Blakeslee (1927) found a mutant form of *Datura Stramonium* in which the number of carpels was three.

Cytological characters, therefore, may be used as a basis of classification in exactly the same way as other morphological characters and are subject to the same restrictions as to universal use. As has been pointed out by Darlington (1937), it is necessary to know the range of variation of characters within a group before they can be used as a basis of classification for the group.

W. W. Smith (1932) has pointed out the way in which cytological

characters can vary within a single genus (*Primula*), and that in some sections of the genus large cytological differences correspond to small morphological differences, whilst in other sections the converse is the case. He does not, however, point out the essential similarity between the type of variation in morphological and cytological characters. Further, he shows that sometimes cytological data fail to uphold morphological relationships and sometimes the converse holds. In either event the resemblances within one or the other set of characters. are most probably due to parallelism. He seems to regard cytological characters simply as additional morphological ones.

Cytologists have tended to go to the other extreme, and to regard cytological characters as of predominating importance, because they are more closely connected with the mechanism of heredity. This is to some extent true, but it should be emphasized that the visible cytological characters are not those most directly concerned in the mechanism of heredity. So that, though differences in chromosome number, size, shape, etc., which are distinguishable characters, may lead to sterility, the carriers of heritable factors are the genes, which are not necessarily altered by such characters.

The characters of chromosome number and chromosome morphology are particularly important as being directly inherited. They can thus often afford direct evidence of the origin of certain forms which cannot be obtained from other characters, whether morphological or cytological (e.g. the origin of such species as *Spartina Townsendii*).

The value of cytological characters as criteria of relationship may therefore be summarized as follows:

(1) They afford additional morphological characters.

(2) They are more intimately connected with the mechanism of heredity.

(3) Some of them are inherited in a more direct manner and can thus sometimes give direct evidence of origin.

GEOGRAPHICAL DISTRIBUTION

Facts of geographical distributions have been used in this paper as confirmatory evidence of phylogeny, and certain assumptions with regard to geographical distribution have been made. These assumptions are in the main based on Willis's (1922) theory of "Age and Area" taken in conjunction with Vavilov's (1926) theory, which is in essence a development of the same idea that species probably originated in the area where they show the greatest variation.

These assumptions are:

(1) If a group is confined to a particular area it probably originated there (e.g. *Tropaeolum*, *Pelargonium*. A less satisfactory case is the Limnanthaceae with few species and very doubtful relationships), unless other considerations show it likely to be a relict group.

(2) If a large group has a well-defined centre of distribution (i.e. a large number of morphologically distinct forms in a comparatively small area) it probably originated there (e.g. *Erodium*, *Oxalis*).

(3) For a widely distributed group (e.g. *Geranium*) geographical distribution can give little indication of its place of origin.

(4) If in such a group as *Geranium* any character is widespread it probably originated early in the history of the group.

Classification

The families considered in this paper are those included in the order Geraniales by J. Hutchinson (1926). These are:

Linaceae
Zygophyllaceae
Geraniaceae
Limnanthaceae
Oxalidaceae
Tropaeolaceae
Balsaminaceae

Earlier writers have used the term Geraniales in a much more extended sense, the two principal classifications, Bentham & Hooker (1862), and Engler & Gilg (1924), including eleven and twenty families respectively, exclusive of those families included by the former under Geraniaceae, though considered separate by Hutchinson.

Bentham & Hooker include in the order all the above-mentioned families, Zygophyllaceae and Linaceae being retained as separate families, the others being united as Geraniaceae, and they include in addition:

Humiriaceae
Malpighiaceae
Rutaceae
Simarubaceae
Ochnaceae
Burseraceae
Meliaceae
Chailletiaceae

Engler & Prantl divide the order into size suborders, of which Geraniineae corresponds roughly with Bentham & Hooker's Geraniales, including all the same families with the exception of Chailletiaceae (called by them Dichapetalaceae) and Malpighiaceae, which are in different suborders, and Ochnaceae, which is placed in the Parietales, and with the addition of Erythroxylaceae and Cneoraceae included in the Linaceae and Simarubaceae respectively by Bentham & Hooker. Finally, and most important for the purposes of this paper, the Limnanthaceae and Balsaminaceae are excluded from the Geraniales and placed in the Sapindales, which differ in the reversed position of the ovule.

Wettstein (1924) includes in the order (which he terms Gruinales) the same families as Hutchinson with the exception of Balsaminaceae, which he places in the Terebinthales, which more or less corresponds with Engler & Prantl's Sapindales, and with the addition of Erythroxylaceae and Malpighiaceae.

All four classifications agree, therefore, in maintaining a relationship between

Linaceae
Zygophyllaceae
Geraniaceae
Tropaeolaceae
Oxalidaceae

but there is considerable difference of opinion as to the proper placing of Limnanthaceae and Balsaminaceae, which are variously regarded, their position varying from inclusion in the Geraniaceae to complete removal from the Geraniales.

With the other families placed in the Geraniales by various authors (see above) the present paper is not concerned.

Various other classifications have been proposed, but the above is enough to indicate the differences of opinion which exist as to the classification of the families included by Hutchinson in the Geraniales.

In the following survey the classification in the main follows that of Engler & Prantl's *Die Natürlichen Pflanzenfamilien*.

SURVEY OF FAMILIES AND GENERA

GERANIACEAE

The most recent monograph is that of Knuth in Engler's *Das Pflanzenreich* (1912). The same classification is adopted by Knuth in the third edition of *Die Natürlichen Pflanzenfamilien*, Knuth's

classification is here followed in the main, though it is open to criticism in some instances on morphological grounds alone. These instances, in so far as they concern the present work, will be discussed in more detail later.

The family is divided into five tribes. Four of these are small, the largest of these (Vivianeae) containing twenty-eight species. Unfortunately, none of these has been available for cytological examination, and they will not be further considered.

The remaining tribe (Geraniae) contains five genera:

Geranium
Erodium
Monsonia
Sarcocaulon
Pelargonium

No species of *Sarcocaulon* have been available for examination. The other genera will now be considered in more detail.

Geranium

(1) *Classification.*

The earliest attempt to divide *Geranium* into sections was made by Koch in 1837. He divided the German species of the genus into three sections:

(1) *Batrachium*, perennial species with an oblique or horizontal rhizome,

(2) *Batrachioides*, perennial species with a fusiform root,

(3) *Columbinum*, annual species.

This has formed a basis for subsequent classifications.

In 1867 Boissier proposed four additional sections, three of these being separated, one from each of Koch's sections and the fourth including some species not found in Germany. These sections each included from one to three rather aberrant species.

The first classification of the whole genus to be attempted was by Reiche (1906). This is based on Boissier's classification with the addition of three sections for species from other parts of the world.

R. Knuth (1903) added two American sections, making twelve in all, and gave a revised key to the sections.

In 1912 Knuth in his monograph of the family for the *Pflanzenreich* increased the number of sections to thirty by dividing up several of the sections of his earlier work. In particular he divided the section *Batrachia* into five sections, and greatly increased the number of

Central and South American sections. As only one South American species has been available for cytological study the present writer does not feel competent to express an opinion on the advisability of the latter procedure. As far as the Eurasian species are concerned, however, the classification is less satisfactory in many respects than the earlier one, resulting, as it does, in the separation of species, which are certainly closely allied morphologically, into distinct sections and the retention of less closely allied species in the same section. Instances of this will be discussed in more detail later.

In what follows, therefore, Knuth's earlier classification has been in the main used with certain modifications based on his later one. Such changes will be specifically mentioned. The order of the sections is such as to be convenient for the present purpose and does not necessarily agree with his.

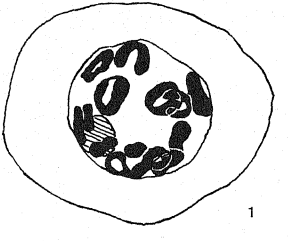
(2) *Cytology.*

Before beginning a detailed account of the species, it will be advisable to give a general account of the cytology. The somatic chromosomes are fairly uniform on the whole, but some striking variations occur in one or two species. In general, the chromosomes are small in size, and more or less uniform within the species. The attachment constrictions are not usually observable, but the shape of the chromosomes shows that they are generally median or submedian. No satellites or fragments have been observed. Size variations between the species are seldom great (except in *G. polyanthes* and one or two other species).

The early stages of the reduction division present no remarkable features. The chromosomes are not easy to distinguish from one another, and these stages will not be considered further. Diakinesis has been clearly observed in several species (Fig. 1). The nucleolus is very clear, and stains nearly as deeply as the chromosomes. The chiasmata are at this stage nearly completely terminalized, but in certain of the species with large chromosomes, chromosomes occasionally occur with an interstitial chiasma in addition to two terminal ones (see Fig. 1) or instead of one of them. Of the configurations that occur, the dumbbell-shaped is the most frequent. A U-shaped configuration also occurs fairly often. Both these have a single terminal chiasma. In nearly all plates examined one or more bivalents showing the ring-shaped formation with two terminal chiasmata occur. The diakinesis configurations have not been studied in more

detail, as the chromosomes are too small for the material to be satisfactory for this purpose.

At metaphase the bivalents appear round in polar view and dumbbell-shaped in side view. The separation of the chromosomes (Fig. 2) shows the chiasmata as a rule to be completely terminalized at this stage. Occasionally, however, configurations with interstitial chiasmata occur. These have been observed in *G. Wallichianum*. The small size of the chromosomes, even in this species, renders more detailed observations even more difficult than at diakinesis. All



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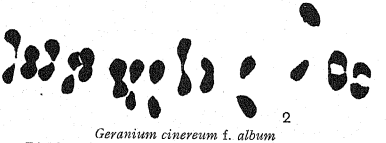

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Geranium cinereum f. *album*

Fig. 1. Diakinesis.
Fig. 2. First division early anaphase spindle view, chromosomes drawn separately.

species of *Geranium*, therefore, fall into Darlington's third group in his "Classification of organisms according to the distribution of chiasmata at metaphase".

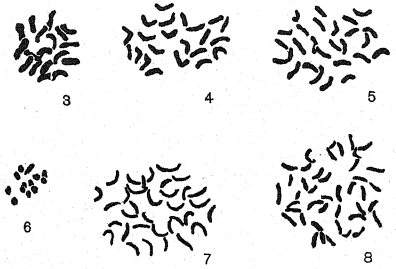
The second division follows a similar course to the first.

No appreciable deviations from this process have been observed in any species of the genus except in the triploid "*G. platypetalum*" of gardens.

Sect. Columbina.

	n	2*n*	Locality
G. columbinum L. (Fig. 3)	.	18	Somerset
G. dissectum L. (Fig. 4)	.	22	Cambridge
	.	22	Coimbra, Portugal
G. molle L.	.	26	Cambridge
	.	26	Aberdeen
	13	.	Lough Ine, Co. Cork
	.	26	Coimbra, Portugal
G. rotundifolium L. (Figs. 5 and 6)	13	26	
G. pusillum L. (Fig. 7)	.	34	
G. deprehensum Almq. (Fig. 8)	.	42	
G. carolinianum L.	.	46 or 48	

The section *Columbina* and the following section, consist of annual species. The chromosome numbers form a marked aneuploid series, but there is no evidence as to what the original number was.



Chromosomes of *Geranium*, Sect. *Columbina*

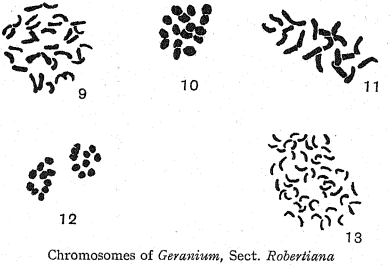
Fig. 3. *G. columbinum* mitotic metaphase.
Fig. 4. *G. dissectum* mitotic metaphase.
Fig. 5. *G. rotundifolium* mitotic metaphase.
Fig. 6. *G. rotundifolium* second division metaphase (one chromosome is seen obliquely).
Fig. 7. *G. pusillum* mitotic metaphase.
Fig. 8. *G. deprehensum* mitotic metaphase.

G. deprehensum and *G. carolinianum*, the two species with the highest numbers, have the most restricted geographical range of the species examined, the former being European only, the latter North American. The other species are all widely distributed in Europe and Asia, and occur in an introduced state in other parts of the world. No variation in number was found within the species when material was examined from different localities.

Sect. **Robertiana** Boiss.

	n	2*n*	Locality
G. lucidum L. (Figs. 11, 12)	10	.	Cambridge
	.	20	Coimbra
G. favosum Hochst.	.	50	
G. Robertianum L.	16	.	Cambridge
G. purpureum Vill. (Figs. 9, 10)	16	.	Britain (loc. uncertain)
	.	32	Montpelier, France

This section is divided into two by Knuth in his later classification, the first two species being placed in a new section *Lucida*. The chromosomes are in general similar to those of section *Columbina*



Chromosomes of *Geranium*, Sect. *Robertiana*

Fig. 9. *G. purpureum* mitotic metaphase.
Fig. 10. *G. purpureum* first division metaphase.
Fig. 11. *G. lucidum* mitotic metaphase.
Fig. 12. *G. lucidum* second division anaphase.
Fig. 13. *G. favosum* mitotic metaphase.

with the exception that those of *G. favosum* (of which only the somatic chromosomes have been examined) are much smaller. *G. Robertianum* and *G. purpureum* are very close morphologically, but *G. lucidum* and *G. favosum* are probably not closely related to these or to each other.

It is noteworthy that in common with section *Columbina* the numbers seem to bear no relation to those of the perennial species.

In Knuth's earlier classification *G. favosum* is placed in section *Columbina*. It is a curious species with cleistogamous flowers and would be perhaps best placed in a distinct section, a view which its very small chromosomes tends to confirm.

Sect. **Batrachia.**

This large section represents what is probably the most primitive morphological type in the genus. Geographically it is widespread in Europe and Asia, extending also to North America. It consists of perennials with large or moderate-sized flowers arranged on long peduncles in few-flowered inflorescences, and a moderately thickened rootstock. In Knuth's later classification it is divided into several sections, as given below. This classification as well as his division into subsections in his earlier classification must be regarded as unsatisfactory.

Sylvatica

	n	2*n*	Locality
G. platypetalum Fisch. & Hey.	.	28	
	.	42	
G. ibericum ialyshense	28	.	
G. Richardsonii Fisch. & H. Trautv.	28?	.	
G. sylvaticum L.	14*	.	
G. eriostemon Fisch.	14	.	
G. pratense L. (Figs. 14, 20)	14*	28	Marlborough, Wilts. (and garden strains)
G. affine Ledeb.	14	28	

Reflexa

G. phaeum L.	14	.	
var. *lividum* Pers.	.	28	Austria
G. reflexum L.	14	.	
G. refractum Edgew. & Hook. f. (Fig. 16)	.	28	
G. sinense R. Knuth	14	.	

Sanguinea

G. sanguineum L. (Figs. 25, 26)	42	84	Cumberland (and a garden strain)
G. napuligerum Franch.	.	28	

Palustria

G. Endressii J. Gay	14	.	
G. palustre L.	14	.	
G. Wlassowianum Fisch.	28	.	
G. collinum Stephan. var. *glandulosum* Ledeb.	.	28	

Striata

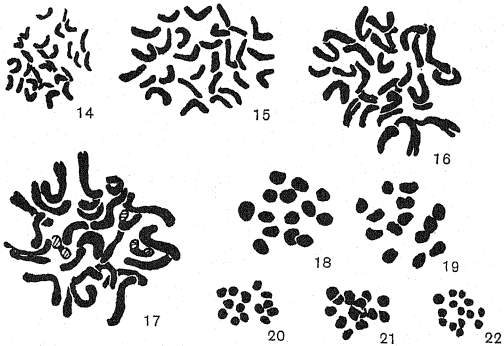
G. striatum L.	14	.	
G. nodosum L. (Fig. 22)	14	.	
G. Wilfordii Maxim.	14	28	
G. Wallichianum D. Don (Fig. 18)	14	.	

* $n=12$ has been given for these two species by Tjebbes (1928). This is considered to be erroneous.

To which are best added the two following species, included by Knuth in this section in his earlier classification, though transferred to Sect. *Pyrenaica* in his later one:

	n	*2n*
G. albanum Marsch.-Bieb.	14	.
G. asphodeloides Burm f.	14	28

In this section, therefore, the majority of species have the haploid number 14. In contradistinction to the annual sections, however,



Chromosomes of *Geranium*, Sects. *Batrachia*, *Polyantha* and *Subacaulia*

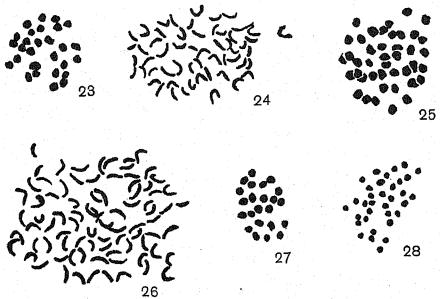
Fig. 14. *G. pratense* mitotic metaphase.
Fig. 15. *G. argenteum* mitotic metaphase.
Fig. 16. *G. refractum* mitotic metaphase.
Fig. 17. *G. polyanthes* mitotic metaphase (chromosomes with shaded ends are curled away from plane of plate).
Fig. 18. *G. Wallichianum* first division metaphase.
Fig. 19. *G. cinereum* f. *album* first division metaphase.
Fig. 20. *G. pratense* second division metaphase.
Fig. 21. *G. albanum* first division metaphase.
Fig. 22. *G. nodosum* second division metaphase.

polyploidy occurs in several species, one triploid, three tetraploids and two hexaploids having been observed. Two of the tetraploids, *G. maculatum* and *G. Richardsonii*, are North American and are the only North American species of this section which have been available for examination.

One of the hexaploid species, *G. sanguineum*, is rather distinct morphologically from the remainder of the section, with its much

dissected leaves and one-flowered peduncles, and bushy habit. Sansome (1936) has shown that this species behaves genetically as an autohexaploid.

The only other species of section *Sanguinea* which has been examined, viz. *G. napuligerum*, has little in common with *G. sanguineum*, being a dwarf alpine plant from China, comparable with the European section *Subacaulia*. The somatic chromosomes are com-



Chromosomes of *Geranium*

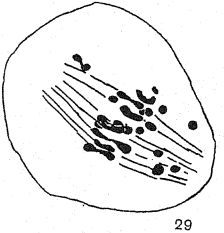
Fig. 23. *G. sessiliflorum* first division metaphase.
Fig. 24. *G. sessiliflorum* mitotic metaphase.
Fig. 25. *G. sanguineum* first division metaphase.
Fig. 26. *G. sanguineum* mitotic metaphase.
Fig. 27. *G. macrorrhizum* first division metaphase.
Fig. 28. *G. anemonifolium* first division early anaphase.

parable with those of *G. refractum* (see below). More of the Asiatic alpine species need to be examined in order to decide whether any affinities exist between these species.

Two of the remaining polyploids, *G. platypetalum* and *G. ibericum talyshense*, fall into a rather well-marked group of Mediterranean species (subsect. *Mediterranea* Knuth) characterized by their rounded leaf-lobes and spreading hairs. The former is a plant widely distributed in gardens under the names *G. ibericum*, *G. ibericum* var. *platypetalum* and *G. platypetalum*. It is vegetatively propagated in gardens. The plant apparently never forms fertile pollen, though the anthers are often apparently well developed. I have never observed seed on the plant, and attempts to make it produce seed by the use of pollen

from other species have been unsuccessful. Morphologically it agrees with the description of *G. platypetalum*.

Counts of the somatic chromosomes show the somatic number to be 42. The reduction division is irregular and is similar in its general



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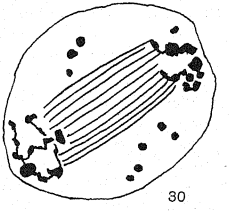

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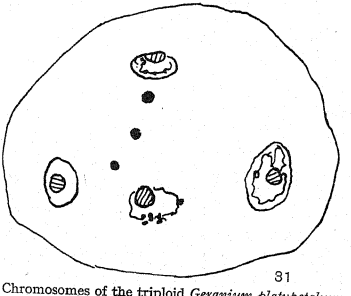

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Chromosomes of the triploid *Geranium platypetalum*

Fig. 29. First division metaphase showing bivalents and univalents.
Fig. 30. Interphase showing univalents not included in either nucleus.
Fig. 31. Early tetrad stage.

characteristics to that described for other triploids. It is difficult to follow in detail owing to its irregularity and the small size of the chromosomes.

At metaphase of the first division the bivalents arrange themselves on the spindle while the univalents remain scattered about the cell, though the greatest concentration is near the median plane of

the spindle. The number of univalents observed or deduced from the total number of chromosomes present appears to vary from 10 to 22, though the higher of these numbers may be too high owing to separated bivalents having been counted as univalents. At interphase some of the univalents are included in the nuclei while others remain scattered about the cell (Figs. 29, 30).

The second division is similar to the first; some of the univalents divide while others remain scattered in the cytoplasm.

Most of the univalents are included in the tetrad nuclei but a few remain in the cytoplasm (Fig. 31).

Cell walls are formed in the normal way, the scattered univalents being included in one or other of the cells and not producing micronuclei.

At this stage development ceases, the tetrads degenerating without producing pollen grains.

It is uncertain whether the plant is an auto- or allotriploid, but the indications point to the latter. No trivalents have been observed, and the number of univalents is larger than is to be expected in an autotriploid. In this connexion also it is noteworthy that the other species of this group (subsect. *Mediterranea*) which have been observed, are diploid and hexaploid respectively. (They are included in the above list under the names with which they were supplied, i.e. *G. platypetalum*[1] and *G. ibericum talyshense*. Neither has yet flowered, so that the correctness of the naming is uncertain, but it is certain from vegetative characters that both belong to this section.) It is not unreasonable to suppose therefore that tetraploids may also occur and that the garden *G. platypetalum* may have arisen as a hybrid between such a form and a diploid.

The remaining polyploid *G. Wlassowianum* is a Central Asiatic plant which may have affinities with this group, though widely separated from it by Knuth. The spreading hairs are similar, but the leaves are much more deeply and sharply lobed.

Certain cytological differences are observable within the group. The somatic chromosomes are in general of the type usual in the genus, but certain differences are observable in *G. refractum* and *G. napuligerum*. The chromosomes of these species are larger, more intricately arranged and lie less flat in one plane. Owing presumably to the larger size, the longitudinal split in the chromosomes is often visible in these species. The meiotic chromosomes of these two species have not been observed.

[1] Not the same plant as the garden *G. platypetalum*.

It is possible to divide the species into three groups according to the size of the chromosomes at meiosis. Group I has chromosomes about 2 μ. in diameter, group III rather more than 1 μ. The groups are not very definite and pass into each other:

I.	II.	III.
Wallichianum	*pratense*	*Endressii*
	affine	*nodosum*
	sylvaticum	*striatum*
	palustre	*asphodeloides*
	albanum	*reflexum*
	Wilfordii	*phaeum*
	maculatum	*sinense*
	platypetalum (triploid)	*Wlassowianum*
		Richardsonii
		sanguineum

These classes are given in descending order of chromosome size.

Chromosome size during meiosis has not been regarded as a very reliable character. Bruun (1932) has objected to its use on the grounds that time of flowering and other external conditions must have an effect.

In the case of *Geranium*, however, it does seem to give some sort of guidance as to possible relationships. Morphologically allied species seem to fall into the same size groups, though it is not suggested that all the species within a size group are necessarily related.

G. Wallichianum, for example, is distinct in this section by its connate stipules, and has little in common with the other members of the section *Striata* in which Knuth places it.

The species of group II are all fairly close morphologically except *G. albanum* and *G. Wilfordii*, which are both rather isolated types among the species examined. *G. palustre* and *G. collinum*, though placed in a different section by Knuth, are not far removed morphologically from *G. pratense*.

Turrill (1928) has pointed out the close relationship between *G. Endressii* and *G. striatum* (again placed in different sections by Knuth); to these *G. nodosum* and *G. asphodeloides* are also probably allied. A second set of closely related species here is composed of *G. reflexum*, *G. phaeum* and *G. sinense*. The other species of this group are rather isolated.

Variations in the fixatives used seem to affect the size little, if at all.

Sect. **Australiensia**

	n	2*b*
G. Traversii Hook. f. var. *elegans*	14	.

This section (Knuth, 1912), which ranges from Java to New Zealand, contains three species, none of which is mentioned by Knuth in 1903. The only species examined, *G. Traversii elegans* has meiotic chromosomes corresponding in size to group II of *Batrachia*. The species is morphologically close to *Batrachia* and would be best included in it.

Sect. **Subacaulia**

	n	2*n*
G. argenteum L.	.	28
G. cinereum Cav. (Figs. 1, 2, 19)	14	.
G. subcaulescens L'Hér.	.	28

This small group of closely allied species is confined to the mountains of Europe and is to be regarded, perhaps, as a development of the last section induced by an alpine habitat. The chromosomes are large, the meiotic ones falling into size group I of the section *Batrachia*.

Sect. **Polyantha**

	n	2*n*
G. polyanthes Edgew. & Hook. f. (Fig. 17)	.	28

Of the three species of this section only one has been available. The section is distinguished by its thick root stock and subumbellate inflorescence.

The somatic chromosomes are the largest observed in the genus and possess the same characteristics as *G. refractum* but in an even more marked degree. Unfortunately the plant died before the meiotic chromosomes could be observed.

Sect. **Andina**

	n	2*n*
G. sessiliflorum Cav. (Figs. 23, 24)	28	56 (2 strains)

Of this large group only one species has been available. This species extends from South America to Australia and New Zealand and is the only member of the section to extend outside South America. In size the somatic chromosomes are like those of the majority of species, and the meiotic chromosomes fall into group III of Sect. *Batrachia* (p. 149). Morphologically the section is characterized by its dwarf perennial habit with thick root, one-flowered pedicels and the absence of a scape, corresponding in these respects with the Chinese *G. napuligerum*.

Sect. **Batrachioides**

	n	*2n*	Locality
G. pyrenaicum Burm. f.	.	28	Cambridge
	.	28	Stockholm, Sweden
	.	28	San Sebastian, Spain

Knuth (1912) also includes in this section *G. albanum*, which is here removed to *Batrachia*. *Geranium pyrenaicum* is remarkable as forming a link morphologically between Sect. *Batrachia* and the annual species of the Sect. *Columbina*, particularly *G. molle*. It is perennial in duration but has a slender descending root and no rhizome, with flowers intermediate in size and foliage rather resembling *G. molle*. The chromosomes are of normal size.

The two remaining sections contain the only two perennial species not forming part of the polyploid series. They are both rather distinct morphologically.

Sect. **Unguiculata**

	n	Locality
G. macrorrhizum L. (Fig. 27)	23	Albania

This species is very isolated both morphologically and in chromosome number. Morphologically it is very distinct in its downward curved stamens, which make the flower zygomorphic, and in its inflated calyx. In size its chromosomes correspond to group III of *Batrachia*. The section contains four species and is entirely Mediterranean in distribution.

Sect. **Anemonifolia**

	n	*2n*
G. anemonifolium L'Hér. (Fig. 28)	34	68

In Knuth's earlier classification it is included in Sect. *Tuberosa* together with some other species with which it has little in common. It is a rather isolated type cytologically. The chromosome number is not easily derived from any other, and the meiotic chromosomes are the smallest observed in the genus. Morphologically it is distinct in its thick unbranched caudex rising above the ground, and terminated by successive years' leaves which are bright green and much dissected. It is probably not long-lived, and rather resembles a large *G. Robertianum* in appearance, though the method of seed formation is different. It is also isolated geographically, being confined to Madeira and the Canaries, in which islands no other perennial species of *Geranium* occur.

(3) *Summary of cytological relations.*

(1) The commonest haploid number in the genus is 14.

(2) Among the perennial species polyploids exist with 14 as basic number.

(3) No annual species occurs with 14 as basic number, the annual species forming an aneuploid series.

(4) Two aberrant perennial species occur with the haploid numbers 23 and 34.

(5) The somatic chromosomes show little variation in size and shape. The extreme types are represented by *G. polyanthes* and *G. favosum*.

(6) In the meoitic chromosomes little variation has been observed. The chiasmata are in general completely terminalized at diakinesis. Certain size variations occur.

(7) Irregularities in meiosis have been observed in one form only, a triploid (*G. platypetalum* of gardens).

(4) *Geographical distribution.*

Before considering the possible phylogeny of the group it will be worth considering the geographical distribution. In general the greatest concentrations of species are in Europe and South-west Asia, China, South America and Mexico, each of these having from thirty-five to fifty species. In addition, about twenty occur in North America and several in the Himalayas, Northern Asia, Tropical Africa and Australia. A very aberrant section occurs in Hawaii. The European, Asiatic and some of the North American species fall in the main into the same sections, and it is mainly these which have been examined: the other regions have mostly endemic sections, some of the Mexican ones extending to the southern United States.

Of the sections examined, *Batrachia* occurs throughout Europe and Asia, extending to North America. It has the widest distribution of any perennial section.

Of the other sections with 14 as basic number *Australiensia* extends from Java to New Zealand, *Subacaulia* is European, *Polyantha* Himalayan and Chinese, *Andina* South American with one species extending to Australia and New Zealand, *Batrachioides*, if confined, as here, to *G. pyrenaicum*, European and Mediterranean.

The two aberrant perennial sections, *Unguiculata* and *Anemonifolia*, are European and Macronesian respectively.

The three annual sections are mainly European in origin though widely distributed by man. Endemic species of *Columbina*, however, occur in North America.

On Knuth's earlier classification representatives of only three sections have not been examined; these three sections are Mexican, South African and Hawaiian.

On the basis of his later classification, however, this number is increased to fourteen, of which six are South American, two Mexican, one Mediterranean, one Eurasian and one Hawaiian.

It is therefore only of the Palaearctic species that at all a representative selection has been examined. For this reason the speculations as to phylogeny which follow must be regarded merely as tentative suggestions.

(5) *Phylogeny.*

The number 14 must be regarded as the primitive chromosome number for the genus. Reasons for this are:

(1) The common occurrence of this number.

(2) The divergence in size of chromosomes of species with this number.

(3) The wide geographical distribution of species with this number.

(4) The possession by species with this number of such presumed primitive morphological characters as perennial habit and actinomorphic flowers.

(5) The number is the lowest found in perennial species.

(6) The formation of polyploids with 14 as basic number.

(7) Seven is the basic number in *Oxalis* and 14 occurs in *Tropaeolum*.

In particular the species of the size groups II and III and with the ordinary type of somatic chromosomes, may be considered to be primitive, since large meiotic chromosomes and larger somatic ones are rare in the genus, and limited in their geographical range.

Various evolutionary lines may be traced from this centre. These are shown in the diagram (1).

Among the diploid species three Asiatic species have noticeably larger somatic chromosomes, namely *G. refractum*, *G. napuligerum* and *G. polyanthes*. The relationship of these three species to each other is very doubtful though the first two may be allied.

Polyploidy must have originated several times in the genus. It is noteworthy that the three American perennials examined are all tetraploid. In the absence of a representative selection of American

species it cannot be stated whether this fact is significant. Besides these species polyploidy probably occurred independently in *G. sanguineum*, and in the *ibericum* group, and must therefore have occurred at least three times, and probably more.

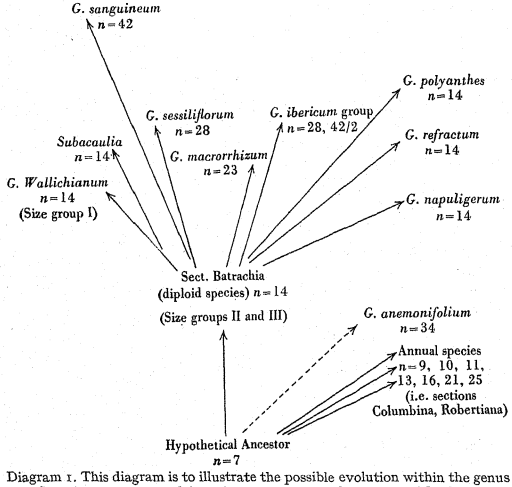

Diagram 1. This diagram is to illustrate the possible evolution within the genus *Geranium* as suggested in this paper, under the section "Phylogeny in Geranium". Size groups refer to the meiotic chromosomes as given on p. 149.

The origin of the annual species is uncertain. Perennials usually precede annuals in evolution, and it is possible that the original annuals were of the *molle-rotundifolium* type, and that the series is to be read in both directions from 13, the number occurring in them. This hypothesis is also suggested by the morphological resemblance between *G. molle* and *G. pyrenaicum*, the closest occurring between any annual and perennial species, taken in conjunction with the fact that *G. molle* has the number 13, which might be obtained from 14 by loss. This loss of chromosomes is only likely to occur if more than one pair of homologous chromosomes occurs in the perennial species.

Fusion of chromosomes is regarded as unlikely owing to the absence of conspicuous size variation within the species.

An alternative hypothesis is that the annuals have originated as an additive series from a species with $n=7$ at an earlier stage in evolution. Both these hypotheses demand the existence at one time or another of a species with $n=7$. Such a species has not been found nor has any species been found which could be a polyploid on 7 but not on 14 (e.g. a species with $n=21$ and regular divisions). Two other possible lines of evidence remain:

(1) The possibility of secondary association of chromosomes at meiosis of those species with $n=14$.

Evidence for this is scanty. There are suggestions of its occurrence in *G. albanum* (Fig. 21), where on some plates one or two of the bivalents seem to be more intimately connected than usual. The same thing has been observed in *G. asphodeloides*.

(2) The possibility of some of the species with $n=14$ behaving genetically as polyploids.

Very little genetical work has been done on *Geranium*, the material being unsuitable, but in hybrids between *G. Endressii* and *G. striatum*, Sansome (1936) has found that certain characters behave in a normal diploid manner.

Evidence for the occurrence of a species with $n=7$ either now or in the recent evolutionary history of the genus is therefore almost entirely negative.

It may be mentioned here that the different groups of annuals are rather distinct morphologically, and may be of independent origin.

The two perennials with aberrant numbers presumably originated from the other perennials, though the small size of the meiotic chromosomes of *G. anemonifolium*, which is only approached by *G. lucidum*, suggest that it may possibly have originated from the annual species, a view which its morphological characters to some extent confirm.

There remains the question of the manner in which the species with high numbers have arisen. The chief possibilities are:

(1) Fragmentation of chromosomes.

(2) Reduplication of chromosomes by various methods.

(3) Hybridization and subsequent reduplication.

The first of these is unlikely to have occurred in *Geranium* as the chromosomes are fairly uniform in size within the species, and this uniformity is not noticeably different in the aneuploids as compared

with polyploids in the origin of which fragmentation is unlikely *a priori* to have played a part.

The two latter possibilities are unlikely to be separable on cytological grounds in a genus such as this where the chromosomes have no marked morphological characteristics.

Hybridization in *Geranium* is rare at the present time, the total number of hybrids recorded being about eight. Several of these are between closely allied species (e.g. *G. Endressii* × *striatum*, *G. argenteum* × *cinereum*). Those between more distantly allied species are rather doubtful; attempts to produce them artificially have so far failed.

The genetic behaviour of *G. sanguineum* is interesting in this connexion. Sansome (1936) has shown that this behaves genetically as an autohexaploid, which suggests that hybridization is unlikely to have played a part in the origin of this species.

Of the various possibilities, therefore, it seems most probable that reduplication is the most important factor in the increase of chromosome number in the genus.

(6) *Comparison with other genera of flowering plants.*

A survey of the cytological types found in the genera of flowering plants has been given by Bruun (1932), and it is not proposed to go into the subject here beyond a consideration of the position of *Geranium* in the scheme and a comparison with other genera of similar cytological type.

Geranium falls into Bruun's class 3, comprising genera showing "more complicated relations". Among these it may be conveniently compared with *Carex* and also with *Viola* in class 2.

Clausen (1929) has produced some convincing ideas on the phylogeny of *Viola*, and drawn up a diagram of the probable origins of the different groups. Though too many important sections in *Geranium* remain unexamined cytologically for this to be done, certain points of similarity between the two genera may be noted. In both cases a basic number is recognizable in the genus. This in *Viola* is found in three of the four main subgenera, in *Geranium* apparently in one central group only, but the sections in *Geranium* are not so clear-cut morphologically as in *Viola*, and the lines therefore are more difficult to follow out. The diversity of number is much greater in *Geranium* than in *Viola*. Hybridity occurs also more commonly in *Viola* than in *Geranium*, and it is probable that evolu-

tion in the latter genus took place at a more remote epoch than in the former.

A special problem is presented by the section *Columbina* with its marked aneuploid series, which forms a parallel to that of the section *Tricolores* in *Viola*. Such additive series are rather rare in plants. Apart from the *Tricolores*, the most striking cases recorded in dicotyledons are in the microspecies of *Erophila verna* (Winge, 1933), and in certain genera of the tribe Madinae, most notably the section *Hartmannia*, of *Hemizonia* (Johansen, 1933). *Celsia*, investigated by Håkansson and given in Tischler's (1931) list, seems to be another example, but I have not had access to the original paper.

In the case of *Crepis* (Babcock & Navashin, 1930), although numerous numbers occur in the genus, closely allied species mostly tend to polyploidy or constancy of number. It seems probable that in this genus such a series may have existed in the past, and that subsequently evolution took place with this series as a basis.

It is possible therefore to trace a succession in which aneuploidy becomes increasingly important as a diagnostic character. Beginning with *Erophila verna* where aneuploidy occurs within the collective species, this succession can be traced through the section *Columbina* of *Geranium*, the subsections of *Crepis* and the sections of *Primula*, to such groups as the *Antirrhinum-Linaria* group, where the different numbers are characteristic of different genera.

From this, one is led to the conclusion that aneuploidy has occurred in different groups at different periods of time, these periods having been followed by periods of stability in this respect. This is in accordance with the conclusions given on p. 135 on the essential similarities between cytological and morphological characters, morphological characters often behaving in a similar way.

It is suggestive in this connexion that all the groups quoted above, where aneuploidy occurs in closely allied species, are annuals or biennials. Annuals in nature are usually found in more or less "open" habitats, where competition is less severe. It is possible that this may enable chromosome types to survive which could not survive elsewhere, and that these types, which at first might be unstable, could become stabilized in such habitats. This applies particularly to *Columbina* and *Tricolores*, both of which sections are mainly ruderals.

Certain monocotyledonous genera should also be mentioned. Heilborn (1928) has found very marked aneuploidy in *Carex* where twenty-four numbers ranging from 9 to 56 occur. In this genus, according to Heilborn, allied species have numbers numerically near

each other, and he suggests that fragmentation is likely to have played an important part in the origin of these numbers. This is not likely to be the case in the dicotyledonous genera given above. *Carex* seems to be unique cytologically, and so far as is known at present, no groups of higher taxonomic value exist which might have originated from such a genus. *Carex* is, however, difficult to reconcile with the idea of open habitats quoted above.

In certain other monocotyledonous genera (e.g. *Ornithogalum*, *Crocus*), there are marked aneuploid series. It seems probable that in these the occurrence of vegetative reproduction and the occurrence of clones accounts for the survival of forms that would otherwise die out because of irregularities in meiosis.

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STATISTICAL STUDIES ON TWO POPULATIONS OF *FRAXINUS*

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(With 2 figures in the text)

In the *Kew Bulletin* (1935, p. 132) an account was published under the title "*Fraxinus Pallisae* and its relationships", in which the known history of this species was given in some detail. It was also pointed out that *F. Pallisae* was morphologically very similar to *F. oxycarpa* Willd. Briefly, it may be recalled that *F. Pallisae* Wilmott (described in *J. Linn. Soc.* (*Bot.*), **43**, 284, 1916) is now known from a number of isolated river deltas in the Balkan Peninsula: Danube, Kamtschija (Kamcija), and Mesta, and in damp soil, especially along river sides, in the Strandja. *F. oxycarpa* is (in the sense in which the species is accepted by Lingelsheim in *Das Pflanzenr.* IV, 243, 53, 1920) a Mediterranean Regional species occurring from Spain, south France, and North Africa, eastwards to Asia Minor, north Persia, and Central Asia. In the Balkan Peninsula it has been recorded from the Ionian Islands, Albania, North and South Macedonia, Thrace, North and South Bulgaria, Dobruja, and Hercegovina. Probably it has a wider and more general distribution than is yet known. Both in ecological and geographical distribution it appears to be less restricted than *F. Pallisae*. Lingelsheim (*loc. cit.*) accepts eight varieties for *F. oxycarpa* differing from one another in the degree of development of the indumentum and wings to the fruits, and in leaflet shape, size, and marginal serration. The only "constant" difference between *F. oxycarpa* s.l. and *F. Pallisae* is in the much more abundant indumentum on the leaflets, leaf rhachis, and young shoots of the latter. The "constancy" of even this character can only be accepted by assuming, on the evidence given below, that intermediates are hybrids.

POLYMORPHISM OF *FRAXINUS OXYCARPA*

It is necessary to consider the polymorphism of *F. oxycarpa* rather more closely. Unfortunately the available material is only of the ordinary herbarium type, i.e. it consists for the most part of single samples, collected from many different populations, and not of sufficient random samples (in the statistical sense) from definite populations. It is, of course, impossible to make a satisfactory study of variation from such material, and the remarks below, while accurate and important as far as they go, are of restricted value because of this limitation. In wild material of *F. oxycarpa* in the broad sense, collected outside the Balkan Peninsula (and thus, so far as is known, without any possible contamination with *F. Pallisae*) the following range occurs in characters of special interest in the present study:

(1) *Leaflet ratio.* Length/width of mature lateral leaflets, from near the middle of the leaf, 1·6 (Afghanistan, *Aitchison* 1081) to 4·2 (Gibraltar, *Wolley-Dod* 1547). On the whole the lowest ratios (relatively broadest leaves) occur to the east of the Balkan Peninsula.

(2) *Pubescence on underside of leaflet.* The majority of the specimens have a narrow belt of hairs on the under-surface of the leaflet on both sides of the midrib in the lower quarter or third. Some specimens, however, are glabrous (Spain, Jaën, *Porta et Rigo*, II, 576; Algeria, *Bourgeau*; Afghanistan, *Aitchison*, 1080, etc.), others have a very few hairs (North Persia, *Gilliat-Smith* 2572) through varying grades to a fairly dense indumentum in the lower half of the under-surface of the leaflet (Crimea, *Callier* 152). Material from the extreme west (Spain and North Africa) and of the extreme narrow type of leaflet seems mainly glabrous. In France, Italy, and to the east of the Balkan Peninsula, the development of the indumentum varies within the above limits. Specimens from the Caucasus (in sylvis ditionis Elisabethpol, *Hohenacker* 1834, and in sylvis prope Schuscha, *Hohenacker* 1839) are particularly interesting because they show a certain amount of indumentum on the leaf rhachis. In the former no branched hairs were found but a few were present in the latter. A specimen from Poland (*Raciborski* 42) also shows hairs on the rhachis. The leaf margins in this specimen are nearly entire and a very few branched hairs were found.

(3) *Serrations at the margins of the leaflets.* In a few specimens the leaflet margins are almost entire (Poland, *Raciborski* 42), usually they are serrated with the serrations 0·5–1·5 mm. long. In a fair

number of specimens the serrations are 2·0 mm. or more in length up to a maximum of 3·0 mm. (Afghanistan, *Aitchison* 1081).

(4) *Number of leaflets per leaf.* In mature normal leaves the leaflets number from 5 to 17 (South France, Restinclières, *Bentham*).

(5) *Channelled petiole and leaf rhachis.* Up to or above the second pair of leaflets, on the adaxial side, this may be narrow (Persia, *Stapf* 1925) or relatively broad and shallow (Tashkent, *Lipsky*).

(6) *Presence or absence of branched hairs.* In leaves with indumentum all, or a considerable proportion, of the hairs, are multicellular and simple. Branched hairs are, however, occasionally present, but are difficult to detect with certainty, without damage to herbarium material. A detailed examination of the under surface of leaflets under a binocular microscope, using alternately high- and low-powered objectives, is usually necessary to distinguish the branching from the overlapping of distinct hairs. It has been impossible to find branched hairs on the majority of the specimens carefully examined in this way. They do, however, occur occasionally (Crimea, *Callier* 152).

(7) *Petiolule length.* The lateral leaflets range from sessile (Smyrna, *Balansa* 381) to petiolulate with petiolules up to 4 mm. long. (Persia, *Stapf.*; Afghanistan, *Aitchison* 1081). Usually the lamina is narrowed down to the point of insertion of the leaflet on the rhachis and it is impossible to say exactly where the petiolule (if any) ends and the lamina begins.

ANALYSES OF TWO RANDOM SAMPLES OF *FRAXINUS*

In species as difficult of interpretation as *F. Pallisae* and *F. oxycarpa*, the biological significance of variation cannot be gauged from individual specimens alone. One needs to know not only the various combinations of characters which may occur, but the comparative frequency of these combinations (Anderson & Turrill, in *Nature, Lond.*, **136**, 986, 1935). For this purpose it is necessary either to visit the region in person or to supplement the customary herbarium specimens with large collections designed to reflect the variation found at a particular place.

We have recently received two collections representing random samples of two *Fraxinus* populations from the Danube (Black Sea) and Mesta (Aegean) Deltas respectively. For these collections and for the great trouble taken in procuring them we have to thank

Dr C. Georgescu and Mr H. G. Tedd. Without their cordial co-operation we could not have prepared this paper.

The Danube Delta collection was first subjected to preliminary analysis, and a considerable range in various characters was easily observed. An initial survey showed that there was correlation between leaflet shape and pubescence, and the simplest hypothesis was to assume that these characters tended to stay together because they had been brought into the population together and there had not been complete fusion of the two (or more) parental combinations. This hypothesis could be tested by determining whether other characters were correlated with those of leaflet shape and pubescence; for every character which was so correlated the explanation became more probable.

From a correlation table for leaflet ratio (length/breadth) and density of pubescence, the specimens were selected which formed the extreme groups, i.e. were at the corners of the table. These were (*a*) those with broad leaflets and more or less glabrous, and (*b*) those with narrow leaflets and dense pubescence. The specimens thus sorted into these two categories were relatively uniform, while those in other combinations were variable. This itself supports the hypothesis. It was now apparent that the leaf margin was more deeply serrated in the (*a*) group than in the (*b*) group. Figure values were then given to the three characters: leaflet shape, pubescence, and degree of serration, and combined into an index with values from 0 to 7. The construction and use of similar indices has been proposed by Anderson in *Ann. Mo. bot. Gdn*, **23**, 511–25, 1936. The names *F. oxycarpa* and *F. Pallisae* are given to these extreme groups in the following remarks. The specimens were resorted according to the preliminary index and carefully examined. It was then evident that the *F. Pallisae* group tended to have a larger number of leaflets than the *F. oxycarpa* group. This was proved by another correlation table. Another index, to include the new character, was made and the specimens again sorted according to it. It was now observed that the *F. oxycarpa* specimens had a strong tendency to have branched hairs and wide channelled petioles. Yet another index was formed by adding on values for these two characters to those already used, with slight adjustments. Another re-sorting indicated that the *F. oxycarpa* leaflets were subpetiolulate at the base and the *F. Pallisae* leaflets rounded and sessile. This character was evaluated in figures and a final index was prepared running from -1 for extreme *F. oxycarpa* to 15 for extreme *F. Pallisae*.

The final index adopted (after testing the Mesta population by the same methods) was based on the following figures:

(1) Leaflet ratio, length/width:

0 = 1·5 − 1·8.
1 = 1·9 − 2·2.
2 = 2·3 − 2·6.
3 = 2·7 − 3·0.
4 = 3·1 − 3·4.
5 = 3·5 − 4·1.
6 = 4·2 − .

(2) Pubescence on under surface of leaflet:

0 = at base of leaflet only.
2 = at base of leaflet and sparsely scattered over surface.
4 = evenly and relatively densely distributed over the entire surface.

(3) Serrations, depth in mm.:

0 = 2 or over. 1 = less than 2.

(4) Number of leaflets per leaf:

0 = less than 11. 1 = 11 or more.

(5) Channelled rhachis, a distinct channel present or absent beyond the first pair of leaflets:

0 = present. 1 = absent.

(6) Branched hairs, leaflets examined under the binocular microscope:

−1 = present. 0 = absent.

(7) Subpetiolule length, in mm.:

0 = 9 − 11. 2 = 3 − 5.
1 = 6 − 8. 3 = 0 − 2.

This index gives:

Minimum score 0 0 0 0 0 −1 0 = −1 (*F. oxycarpa*).

Maximum score 5 4 1 1 1 0 3 = 15 (*F. Pallisae*).

The results of summating the values for the two populations can be expressed in the form of two frequency polygons. These show some resemblances to and some differences from one another. A contrasting table will emphasize these resemblances and differences:

	Danube Delta	Mesta Delta
Number of individuals in sample	167	47
Number of modes	2	2
Position of modes	4–5, 10–13	6–7, 12–13
Index range	−1 to 15	2 to 15

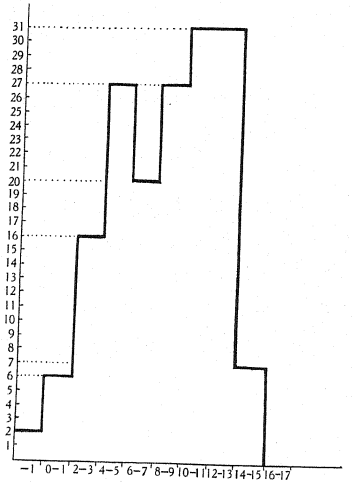


Fig. 1. Frequency polygon based on the sum-totals of values given for selected characters in a sample of 167 plants from a population of *Fraxinus* growing in the Danube Delta.

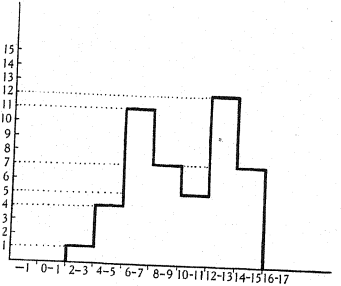


Fig. 2. Frequency polygon based on the sum-totals of values given for selected characters in a sample of 47 plants from a population of *Fraxinus* growing in the Mesta Delta.

The sample from the Danube Delta, on the whole, shows rather more extreme *F. oxycarpa*, and that from the Mesta Delta rather more extreme *F. Pallisae* characters. Before discussing this matter in greater detail it is advisable to consider the distribution of the characters separately in the two populations. These may be tabulated as follows:

Leaflet ratio:

	1·5–1·8	1·9–2·2	2·3–2·6	2·7–3·0	3·1–3·4	3·5–4·1	4·2–
Danube Delta	10	38	43	37	29	10	0
Mesta Delta	1	0	9	13	11	10	3

Pubescence:

	Limited (0)	Scattered (2)	Dense (4)
Danube Delta	61	15	91
Mesta Delta	22	3	22

Serrations:

	Less than 1 mm.	1–1·9 mm.	2 or more mm.
Danube Delta	29	51	87
Mesta Delta	10	32	5

Number of leaflets:

	5	7	9	11	13	15
Danube Delta	5	14	50	58	30	10
Mesta Delta	0	3	13	21	8	2

Branched hairs:

	Present (−1)	Absent (0)
Danube Delta	22	145
Mesta Delta	25	22

Channelled rhachis:

	Present (0)	Absent (1)
Danube Delta	133	36
Mesta Delta	31	16

Petiolular length:

	1 mm. or less	2 mm.	3 mm.	4 mm.	5 mm.	6 mm.	7 mm.	8 mm.	9 mm.	10 mm.	11 mm.
Danube Delta	28	36	30	30	17	5	7	6	4	3	1
Mesta Delta	21	13	4	6	2	1	0	0	0	0	0

DISCUSSION OF CHARACTER DISTRIBUTION IN SPECIES OF *FRAXINUS* POSSIBLY CONNECTED WITH THE TWO POPULATIONS

The extreme type of relatively broad leaflet is much more strongly represented in the collection from the Danube Delta than in that from the Mesta Delta. This is so marked that it is necessary to consider the possibility of three or more and not two species being involved in the populations. *F. Pallisae* from both areas is relatively

uniform or, at least, only shows a slight degree of fluctuation which may well be considered intraspecific. *F. oxycarpa* in the broad sense (e.g. as the species is accepted by Lingelsheim) is, on the other hand, acknowledgedly polymorphic in leaf-shape. The original descriptions of *F. oxycarpa* (Willd. *Sp. Pl.* **4**, 2, 1100, 1806) and of its synonym *F. oxyphylla* (M. Bieb. *Flor. Taur.-Cauc.* **2**, 450, 1808) give the leaflet-shape as lanceolate. The variety *parvifolia* (Lam.) Wenzig was originally described as a species by Lamarck (*Encyl.* **2**, 546, 1786), and the leaflets were said to be ovate. In this species (or variety of *F. oxycarpa*), however, the leaflets seem to be always of small size. *F. rotundifolia* Lam. (*Encyl.* **2**, 546, 1786) is now generally regarded as a variety of *F. Ornus*). The leaflets are described as obovate, very minutely denticulate, unequal at the base, with the terminal more obtuse. Except in leaf-shape this description does not fit any of the leaves in the Danube and Mesta populations. As pointed out above, the broad leaf character is strongly correlated with long marginal teeth and the leaflets are not (or rarely) unequal at the base. On the other hand, some cultivated specimens, preserved at Kew under the names *F. rotundifolia* and *F. Ornus*, are remarkably similar in leaflet-shape and serrations to our extreme broad-leaved type. The suggestion that *F. Ornus* in a "*rotundifolia*" variation has taken part in producing the mixed populations cannot be entirely dismissed, but on general grounds seems unlikely. Another species requires mentioning here. This is *F. potamophila* Herder in *Bull. Soc. Nat. Moscou*, **41**, 1, 65, 1868, from Turkestan. It is, in particular, similar to the broad-leafleted Danube and Mesta Delta plants by the often ovate leaflets, with distinct petiolules, and sometimes with coarse serrations. There is, judging from the original description and the few specimens available, a considerable range even in these characters. Herder describes the petiolules (he calls them petioles) as 4–6 cm. long, but in specimens at Kew they are sometimes much shorter than this and no longer than in some of our specimens from the Balkan Peninsula. The texture, however, is thicker and the details of the venation are not quite the same, though, from the poverty of the available material, it is difficult to evaluate these differences.

F. obliqua Tausch. (in *Flora*, **17**, 2, 521, 1834), which, according to Rehder (*Manual of Cultivated Trees and Shrubs*, p. 748, 1927) has *F. Willdenowiana* Koehne (*Deutsche Dendro.* p. 515, 1893) as a synonym, can also be approximately matched in our Danube and Mesta samples. Rehder states (*loc. cit.*) that it comes from West Asia, but most authors (e.g. Elwes & Henry, *Trees of Great Britain*

and Ireland, **4**, 884, 1909, under *F. Willdenowiana*) record that its native home is unknown. Tausch says "Stammt wahrscheinlich aus Nordamerika", and contrasts it with *F. parvifolia* W., noting that it has "leaves" double the size of this with a conspicuous ovate base, not so long and cuneate. Elwes & Henry comment on the large terminal leaflet and grooved rhachis.

F. syriaca Boiss., *Diagn.* I, **11**, 77 (1849), is kept as a distinct species by Lingelsheim (*Pflanzenr.* IV, 243, 53, 1920) though it is reduced to a variety of *F. oxycarpa* (as var. *oligophylla*) by Boissier, *Flor. Or.* **4**, 40, 1875, by Wenzig in *Engl. Bot. Jahrb.* **4**, 175, 1883, and by other authors. It is essentially distinguished by having only 1–3 leaflets, but it is doubtful if this character is very constant. Lingelsheim (*loc. cit.*) quotes specimens from Aetolia, Euboea, and near Constantinople. Specimens named *F. syriaca* (or *F. oxycarpa* var. *oligophylla*) in herbaria sometimes approach specimens in the Danube and Mesta populations in having relatively broad leaflets and coarse serrations.

Having surveyed the species, other than *F. oxycarpa*, which might possibly be connected with our populations, it must be recorded that the leaflet shapes of *F. oxycarpa* in the broad sense probably cover the leaflet shapes of the material analysed from the Danube and Mesta Deltas. While the majority of the herbarium specimens of *F. oxycarpa* examined range in leaflet shape from lanceolate to narrow lanceolate, a few have ovate or broadly elliptic leaflets (e.g. Persia: Bagibagsh bei Schiras, *Stapf.* 1925; Afghanistan, *Aitchison* 1081). These broader leaflets have also coarse serrations and are petiolulate. Boissier (*Diagn.* II, **3**, 119, 1856) describes *F. petiolulata* from the Cicilian Taurus (Kotschy 356 *a*). In the *Flor. Or.* **4**, 40, 1875 he reduces this to a variety of *F. excelsior* L., with the remark, however, "varietas ob foliola magna lata 3½–4 pollices longa huc potius quam ad formas *F. oxyphyllae* referenda". The specimen has not been seen but it may possibly be the broad-leafleted variety or form of *F. oxycarpa* (syn. *F. oxyphylla*), though it is reduced by Lingelsheim (*Pflanzenr.* IV, 243, 18, 1920) to *F. ornus* L. var. *juglandifolia* Ten., a classification supported by Boissier's description "foliolis...margine obsolete crenulatis".

F. oxycarpa var. *aspera* (*F. oxyphylla* var. *aspera* Podp. in *Verh. zool.-bot. Ges. Wien*, **52**, 664, 1902) is described in the words "foliola ovata vel ovato-orbiculata, argute dentata, cum rhachide pilis albis rigidiusculis aspera. Parva frutex". The type was from Harmanlij.

It is possible (even probable) that some of the trees from which

the broad leaflets in our samples were obtained were immature specimens or, still more probable, had been pollarded or in other ways cut back. Pollarding and the cutting off of lateral branches is very common in the Balkan Peninsula and is recorded for the ashes of the Mesta Delta (*Kew Bull.* 1935, pp. 134–7). Further, pruning, pollarding, or general cutting back causes leaf abnormalities of various kinds in *F. excelsior* (see Sprague in *Proc. Linn. Soc. Lond.*, 135th Session, p. 4, 1923). There is also some evidence from herbarium material that a considerable difference in leaflet shape and other characters can occur between the leaves on barren and on fruiting branches.

Pubescence in *F. oxycarpa* has been considered above, and it is only necessary here to remark that no other known species appears to be responsible for dense indumentum in the Danube and Mesta populations than *F. Pallisae*. The figures from the analysis of the two samples suggest strongly that pubescence gives the clearest character for separating two types, and this is the main taxonomic character for the separation of *F. oxycarpa* from *F. Pallisae*.

Extremely developed serrations in herbarium specimens of *F. oxycarpa* are usually correlated with greater leaflet width. The development known in this species is quite sufficient to cover the whole range in the Danube and Mesta populations. While specimens determined (chiefly on indumentum characters) as *F. Pallisae* have sometimes inconspicuous teeth, they sometimes show them quite well developed (e.g. *Tedd* 1322). The rather irregular distribution of this character is indicated in the figures given for the two population samples.

The number of leaflets per leaf shows for both populations one mode at 11. In series of herbarium specimens of both *F. oxycarpa* and *F. Pallisae* there is a considerable range in this character and its taxonomic value is doubtful. Wilmott in the original description of *F. Pallisae* gives the leaves as "5–9 (−11) foliolata" (*J. Linn. Soc. (Bot.)*, **43**, 284, 1916).

The presence or absence of branched hairs is an unsatisfactory character for scoring. They may be present in such small numbers that their detection amongst the more numerous, and sometimes dense, simple hairs is difficult. They are easily overlooked. Since they do not appear to occur in clear-cut *F. Pallisae* and only rarely in *F. oxycarpa*, judging from the available herbarium material, their value is difficult to estimate. It is most probable that they entered the Danube and Mesta populations with *F. oxycarpa*.

A channelled rhachis is present in the majority of plants in both samples. In *F. oxycarpa* it is most often well marked, and in *F. Pallisae* absent or obscure.

The development of a petiolule is strongly correlated in general herbarium specimens of *F. oxycarpa* with greater width of leaflets and coarser serrations—as it is in the mixed Danube and Mesta Delta samples. The range in these latter is indeed covered by the known variation within accepted *F. oxycarpa*. It must, however, be noted that "typical" *F. oxycarpa*, like typical *F. Pallisae*, has practically sessile leaflets. It is difficult to measure the petiolule because, if it be present, the leaflet lamina usually narrows into it gradually. In analysing the samples an arbitrary line of demarcation had to be taken, but this was done uniformly. The Danube sample had an average petiolule (or subpetiolule) length of 3·6, and the Mesta sample one of 2·1. This is undoubtedly correlated with the greater extreme *F. oxycarpa* influence in the Danube sample.

CULTURAL DATA

F. oxycarpa is not infrequent in cultivation. According to Elwes & Henry (*Trees of Great Britain and Ireland*, **4**, 882, 1909) typical *F. oxycarpa* is "much rarer in cultivation in England than *F. angustifolia*". The latter is now frequently regarded as a variety of *F. oxycarpa*, but is not further discussed here, since it is unlikely that, if it be really distinct from *F. oxycarpa*, it enters into the populations with which we are immediately concerned.

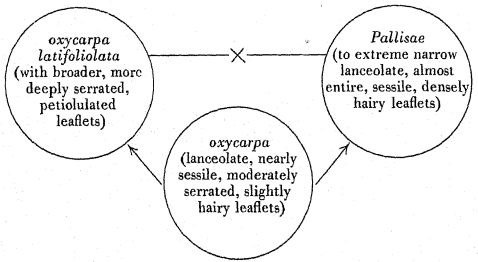
One interesting result has been obtained from cultivated material. A packet of seed from the Strandja Planina was received from Prof. B. Stefanoff in 1932, under the name *F. Pallisae*. This was sown at Kew and four young saplings about 1 m. high were in cultivation from it in the Aboretum Nursery on 20. ix. 1935. The leaflets of these young plants cannot, of course, be said to have a shape typical for the adult plants—actually the shape recalls that of *F. parvifolia*, and supports the suggestion that some herbarium specimens called *F. parvifolia* may represent juvenile conditions. However, all four saplings are different in indumentum characters: No. 1 has the leaflets with hairs restricted to the lower surface, and a few branched hairs are present; No. 2 has the leaflets glabrous or with a few hairs on the underside near the base, and a very few of these are branched; No. 3 has scattered hairs on the under surface, but no branched ones have been detected. No. 4 has the leaflets densely

hairy on the lower surface, no branched hairs were seen, but scattered hairs occur on the upper surface. This is further evidence of hybridization, though in another area than the Danube and Mesta Deltas.

Conclusions

The general polygons obtained for the Danube and Mesta samples suggest from their bimodal outline that two species are involved, and that though much mixing has taken place since the two met they still tend to maintain their identities. That one of these species has to bear the name *F. Pallisae* seems certain, and that the other is *F. oxycarpa sensu lato* (e.g. that of Lingelsheim) is probable. There is, however, the difficulty of accounting for the extreme broad-leafleted plants. This difficulty may be more nomenclatural than real. Typical *F. oxycarpa* (i.e. var. *oxyphylla* (M. Bieb.) Lingelsh.) has lanceolate leaflets and is not only the nomenclatural type but also the most widespread type. From the available material and the published literature it seems not improbable that *F. oxycarpa* can, either as one or more genetical varieties or as forms (due to pollarding or other treatment), produce relatively broad leaflets. Taking all the known facts of variation and distribution into account it seems a reasonable hypothesis to assume that *F. oxycarpa* has given rise here and there over a wide distributional area, but especially in the east, to broad-leafleted plants (conveniently termed *F. oxycarpa latifoliolata*) and, locally in some Black Sea and Aegean river deltas, to a hairy-leaved plant (*F. Pallisae*). In the Danube and Mesta Deltas these plants have hybridized with the formation of hybrid swarms, of which two samples have been analysed for leaf characters. The two hybrid populations, while agreeing in their main features, differ somewhat as to details, one being on the average more like *F. oxycarpa*, the other more like *F. Pallisae*. It should be emphasized that this result is exactly in accord with theoretical expectations since the end result of hybridization between two species will depend, among other things, on the relative frequencies of the original species and the effect of selection upon the hybrids. Any two such widely separated areas as the Danube and Mesta Deltas would therefore be expected to show somewhat different results from hybridization between the same two species. If this hypothesis be correct then it is simplest to assume that *F. Pallisae* arose independently in the Aegean and Black Sea areas and is "younger" than *F. oxycarpa*. This hypothesis is in accord with what is known of the geological history of the

areas and with the habitat preferences of *F. Pallisae*. It is, however, impossible to be certain from the data at present available that the following diagram correctly represents the history of the Mesta and Danube populations.



APPENDIX

Note on the anatomy of Fraxinus oxycarpa *and* F. Pallisae

It is well known that the wood anatomy of species of *Fraxinus* is extremely similar and that it is difficult or impossible to distinguish one species from another by wood structure. It is, therefore, not surprising that an examination of 5-year-old stems of *F. oxycarpa* and *F. Pallisae*, collected in the Mesta Delta by H. G. Tedd, show no anatomical characters by which they can be differentiated. Petiolar structure also shows no constant differences separating the two species, except that unicellular hairs are present in *F. Pallisae* and absent in *F. oxycarpa*. These hairs have rather thick walls, are somewhat swollen at the base, and taper to the apex. The epidermal cells surrounding the hairs are rather enlarged and often show granular contents. The hairs themselves are to be regarded as specialized epidermal cells with thickened walls and filled to a greater or less extent with granular material. C. R. METCALFE.

ON THE UPWARD INHIBITING EFFECT OF AUXIN IN SHOOTS

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(With 2 figures in the text)

1. Introduction

In a recent paper in this periodical Le Fanu (1936) reported that a ring of a paste of lanoline containing hetero-auxin at concentration 1 in 2×10^4,[1] when placed round a young internode of a pea seedling, growing in the light, strongly inhibited the elongation of the next younger internode above, which was from 3 to 5 mm. long at the start. Even a hetero-auxin paste of concentration only 1 in 2×10^5 inhibited the same young internode considerably. She also found that a paste of 1 in 10^5, when applied to the top upper cut surface of a pea seedling, decapitated at the top of a young internode about 3 mm. long and growing in the dark, strongly accelerated the growth of that internode. She reached the conclusion (p. 212) that hetero-auxin paste of a strength capable of partially inhibiting the growth of young internodes when applied to a part of the stem morphologically below them, accelerates the growth of similar internodes when applied to their upper ends.

The writer (1936), using a hetero-auxin paste of 1 in 2000, found in confirmation that a ring of this paste placed round a young pea internode in the light, inhibited and finally arrested completely the growth of the younger stem parts above. He added the points that the very young leaves of the terminal bud are also arrested (those of length 1·5 mm. and over were removed), and that the inhibition is preceded by an acceleration lasting for several hours.

Recently Pohl (1937), using a paste of the sodium salt of hetero-

[1] Le Fanu (1936), and at one time the writer (1936), expressed the concentration of their hetero-auxin paste as the ratio of the hetero-auxin to the water which, together with an equal quantity of wool fat, made up the paste. But it is more usual to express it as the ratio of hetero-auxin to total paste, and consequently the previous statements of concentration are here translated to this basis.

auxin, found that the upper zones of oat coleoptiles were inhibited by a ring of paste put round their middle or lower zones. The same ring of paste accelerated the growth of the zones below it, so that his results support those of Le Fanu. It does not appear that the upward inhibitions were well marked until after 24 hr. or longer, so that possibly a search after shorter periods would reveal upward accelerations, such as were found after short periods by the writer (1936) in oat coleoptiles as well as in peas.

On the other hand, Nagao (1937) has criticized Le Fanu's conclusion on the ground that her plants with paste on top were decapitated, whereas those with paste below were not. He considers that the retardations in her experiments were due to an excessive concentration of auxin, and that the total concentration was higher when the paste was applied below because then the applied hetero-auxin was added to the natural auxin coming down from the leaves.

But against this explanation it may be objected:

(1) That the hetero-auxin pastes used by Le Fanu were actually very weak. (It must be remembered that hetero-auxin in lanoline needs to be about 1000 times more concentrated than in agar in order to be equally effective.)

(2) That in an experiment which Nagao has quite overlooked, Le Fanu found that the upward inhibiting effect of a paste of 1 in 2×10^5 was much greater if the young leaves of length 5 mm. and over were removed, than if they remained. Since these leaves still form plenty of auxin, this is just the opposite of what would be expected on Nagao's theory.

(3) That in the writer's experiments (1936), mentioned above, the young leaves of length 1·5 mm. and over were removed from the terminal bud. Since the remaining still younger leaves form very little auxin (compare Snow, 1929), this operation is physiologically almost equivalent to decapitation.

However, Nagao has admittedly pointed out an imperfection in Le Fanu's evidence for her conclusion, and another imperfection is that her experiments with the paste below were performed in the light, and those with the paste above in the dark. In the present paper, therefore, some experiments will be reported which are not open to these objections, and also some further experiments which help towards interpreting the upward effect of auxin paste.

2. Methods

The hetero-auxin used for the experiments, which was obtained commercially, was found, when tested on coleoptiles, to be about 2·5 times less effective than the hetero-auxin which was synthesized by Dr R. Weissberger at Oxford and used previously by Le Fanu (1936) and the writer (1936): consequently allowance must be made for this difference. The experiments were all performed on pea seedlings (Thomas Laxton), from seed sown unsoaked in soil, and growing in the shade in a greenhouse or frame. The term "auxin" will be used to include both the natural auxin (which may be auxin-*a*) and also the applied hetero-auxin, which acts similarly in nearly all respects.

3. Comparison of effects of auxin paste applied above and below

In a first experiment rings of hetero-auxin paste, of concentration 1 in 2000, were placed round the upper ends of internodes of pea shoots, when the next younger internodes (the 5th from the epicotyl) were from 2·5 to 4 mm. long. The growing leaves, including all those above the paste, were removed, and the shoot was decapitated rather close beneath the apex. Controls were treated similarly except that they were not given a ring of paste. Any buds which grew out were removed. Table I shows the results.

Table I. 1 *in* 2000 *paste below*

	At start, length in mm. of 5th internodes	After 12 days: 5th internodes	After 12 days: 6th internodes
Experiments	4	32	3
	3	24	5
	5	26	14
	2·5	24	9
Mean	3·6	27	8
Controls	2·5	90	60
	3·5	88	51
	3	76	32
Mean	3	86	48

Table I shows that the young internodes just above the 1 in 2000 paste grew much less than the corresponding internodes in the controls, and that the next younger internodes, which at the start were too small to measure, were still more strongly inhibited, though

not quite completely arrested. Decapitation therefore does not prevent an auxin paste from retarding the young zones above it.

A similar experiment, Exp. 2, was performed with a stronger paste, 1 in 600. The results are given in Table II.

TABLE II. 1 *in* 600 *paste below*

	At start, mean length in mm. of 5th internodes	After 6 days, 5th internodes	After 8 days	
			5th internodes	6th internodes
Experiments (4 plants)	6·4	25	27	<2, mostly dying
Controls (3 plants)	6·8	71	81	40

Table II shows that the 1 in 600 paste inhibited the parts above even more strongly. The internodes next but one above the paste were completely arrested at lengths of less than 2 mm., and in three out of four plants they were dying after 8 days.

It was next necessary to compare the effects of the same pastes when applied from above. For this purpose the pea seedlings were operated on in just the same way and the pastes were applied to the upper cut surfaces of the stem, which were close above the corresponding young measured internodes but separated from them by two or three nodes. After 2 or 3 days, and again after 4 or 5 days, the paste was removed and replaced with fresh paste. The amounts applied were as large as could be applied without their overflowing more than a very little way below the upper cut surface. Controls were similarly operated on, but had pure lanoline applied to them instead of auxin paste. The seedlings were younger by about two plastochrons than in the previous experiments, but the measured internodes were of about the same length at the start. The results of an experiment (Exp. 3) with the 1 in 2000 paste are given in Table III. The figures given in brackets after the mean lengths show the numbers of plants on which the mean is based. The means for the youngest (5th) internodes are based on fewer plants because in some of the plants these internodes were cut through in decapitating.

TABLE III. 1 *in* 2000 *paste on top*

	At start, mean length in mm. of 3rd internodes	After 8 days		After 11 days		
		3rd internodes	4th internodes	3rd internodes	4th internodes	5th internodes
Experiments	3·8 (6)	56 (6)	23 (6)	56 (6)	35 (6)	18 (4)
Controls	3·9 (6)	50 (6)	21 (6)	54 (4)	50 (4)	16 (3)

The results of a similar experiment with the stronger paste, 1 in 600, are given in Table IV. The standard deviations of the means, σ_m, are given in brackets where they seem needed.

TABLE IV. 1 *in* 600 *paste on top*

	At start, mean length in mm. of 3rd internodes	After 8 days		After 12 days		
		3rd internodes	4th internodes	3rd internodes	4th internodes	5th internodes
Experiments	4·2 (6)	45 (6)	25 (6)	46 (6)	28 (6) ($\sigma_m = 1{\cdot}58$)	16 (4) ($\sigma_m = 2{\cdot}62$)
Controls	4·2 (6)	42 (6)	31 (6)	42 (5)	50 (5) ($\sigma_m = 2{\cdot}09$)	21 (5) ($\sigma_m = 2{\cdot}05$)

In Table III it can be seen that the 1 in 2000 paste (Table III), applied above, had no appreciable effect on the elongation of the young 3rd internodes, which were about 4 mm. long at the start, nor on that of the two next younger internodes (the difference between the mean lengths of the 4th internodes after 12 days is not significant). Yet the same paste when applied below inhibited the elongation of similar young internodes very strongly. The 1 in 600 paste applied above (Table IV) did not affect the elongation of the young 3rd internodes either, but it did a little diminish the final length of the next younger internodes, which at the start were too small to measure. But this inhibition was very slight in comparison with the inhibition caused by the same paste when applied below (Table IV): for applied below it completely arrested the corresponding young internodes at lengths of under 2 mm. and finally killed them.

The difference between the effects of the pastes applied above and below showed itself conspicuously also in the growth in thickness of the young internodes. For even the weaker paste, when applied above, caused all the young internodes to swell (that is, to grow in thickness) until they were thicker than those of the controls or of intact plants. The stronger paste, applied above, made them all swell even more strongly, including the very youngest internodes, of which it diminished the elongation. On the other hand, when the pastes were applied below the young internodes, the swellings which they caused in the zones to which they were applied did not extend more than a little way up the next internodes above, which were a few millimetres long at the start. They never extended to the tops of these internodes, nor into the still younger internodes, which were the most strongly inhibited of all. Indeed these younger internodes

remained thin and weak, and when the paste was the stronger one, they turned yellow and died, just like shoots that are inhibited correlatively.

Although, therefore, in the present experiments the auxin pastes which inhibited when applied below did not increase elongation when applied above, yet the results, besides refuting Nagao's proposed explanation, confirm the most important element in Le Fanu's discovery; that is, that auxin paste applied morphologically below the young internodes exerts an inhibiting effect upon them which is quite different from any effect which it exerts when applied above them.

It may perhaps be suggested that the pastes applied below acted differently from the same pastes applied above because the plant absorbed more auxin when the paste was applied below: for the area of surface to which it was applied was then much greater. But this difference was probably much outweighed by the fact that in stems of peas and beans the auxins are transported much more effectively in the morphologically downward than in the upward direction (Thimann & Skoog, 1934, p. 337; Le Fanu, 1936, p. 216): for otherwise it would be difficult to understand how it was that the youngest internodes swelled when the paste was applied above, but not when it was applied below.

The effect on stem elongation of a source of auxin applied above will probably depend in general on the concentration of the applied auxin and on the amount of natural auxin present in the stem, as well as on the physiological age of the zone of stem considered. For Thimann & Skoog (1934, p. 331) found that in decapitated shoots of *Vicia Faba* agar blocks containing a rather high concentration of auxin, slightly retarded elongation when applied above, provided that the leaves, which also supply auxin to the shoot, were allowed to remain. But if the leaves were removed, then the same amounts of auxin, similarly applied, accelerated elongation; and in plants grown in the dark, which contain much less natural auxin, they did so much more strongly still (p. 333). This agrees with Le Fanu's finding (1936) that her weak auxin paste, applied to the tops of decapitated pea shoots growing in the dark, increased elongation, and also with Nagao's interpretation of his own experiments on sunflower hypocotyls (1937).

It should be mentioned that some workers have inhibited the elongation of shoots by applying strong auxin paste all along the elongating zone: in such experiments the different effects of applying

the paste above and below cannot be separated. Others again have added auxins to the soil in which plants were rooted and have then measured the growth of the shoots, with differing results. For the purpose of learning more about the effects of auxin on shoot growth, this last method of experimenting is not very helpful, since the resulting conditions are much too complicated. For the growth of the shoots may be affected both secondarily as a result of the action of the auxin on the roots, and also by auxin drawn up with the transpiration stream; and the effects of auxin in the transpiration stream are themselves in turn complicated and different for different parts of the shoot (see Snow, 1936, p. 300; 1937, p. 296).

4. Experiments to test the nature of the upward inhibiting effect

The following experiment (Exp. 5) helps towards understanding the nature of the upward inhibiting effect. It is illustrated in Fig. 1, in which figure, as also in Fig. 2*a*, *b*, the younger parts of the shoot are drawn unnaturally large so as to show more clearly.

Young pea seedlings, grown in summer, had their main roots split longitudinally, when still quite short, in the plane of the leaves; the split was continued up through the cotyledonary node and the seedlings were replanted. Subsequently the split was continued by stages up the shoot as it elongated, the cut surfaces above ground being vaselined, until the split reached the base of the young fourth internode. Finally this internode was split very carefully with a fine scalpel when it was from 5 to 11 mm. long, its cut surfaces were washed, and a large ring (larger than in the previous experiments) of hetero-auxin paste, 1 in 600, was placed round the upper end of one of the halves of the next internode below. The shoots were decapitated close beneath the apex, the exposed cut surfaces were covered with vaseline, and all leaves were removed. Controls were treated similarly, except that they did not receive any paste. The plants were kept in rather damp air. Table V shows the lengths of the halves of the young fourth internodes after 9 days from the final operation, and also the lengths of the next younger (5th) internodes, which were too small to measure at the start.

The results (Table V) show firstly that the ring of auxin paste strongly inhibited the elongation of both halves of the young split internode just above it, although in the farther half of this internode any substance or influence coming from the paste must have been

travelling in the morphologically downward direction (see Fig. 1): indeed, these farther halves were inhibited very nearly as strongly as the halves immediately above the paste (the lengths of the halves

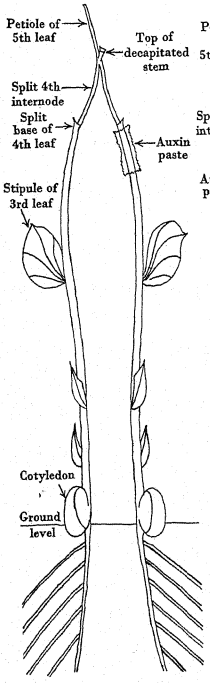

Fig. 1.

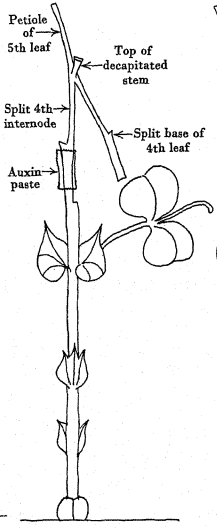

Fig. 2*a*.

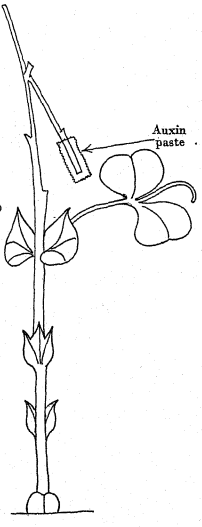

Fig. 2*b*.

given in the table are the final lengths, or nearly so). It follows, therefore, that the inhibiting effect of auxin paste on the young parts morphologically above it, does not depend on the morphological

TABLE V. *Exp.* 5 (*see Fig.* 1)

			After 9 days		
		At start, length in mm. of 4th internodes	4th internodes		5th internodes
			Half above paste	Farther half	
Experiments:	No. 1	9·0	18	24	11
	2	8·5	15	21	16
	3	11·0	20	23	8
	4	6·0	18	23	20
	5	6·5	19	24	13
	6	5·0	10	10	3
	Mean	7·7	16·7 $\sigma_m = 1{\cdot}37$	20·8 $\sigma_m = 2{\cdot}00$	11·8
			Two similar halves		
Controls:	No. 1	6·5	25 and 30		30
	2	10·0	28 and 31		30
	3	8·0	22 and 22		20
	4	6·0	35 and 35		38
	5	7·5	39 and 41		43
	6	5·5	37 and 37		24
	Mean	8·25	31·8 $\sigma_m = 0{\cdot}68$		30·8

direction in which any substance or influence coming from that paste may be travelling. A similar conclusion was reached previously by the writer (1932, p. 87; 1937, p. 296) with regard to correlative inhibition, by means of an experiment with similarly split young internodes; but the present experiment is more satisfactory since it gives to each of the split halves a direct connexion with roots and cotyledon below, which the previous experiment did not.

Secondly the results show that the upward inhibiting effect of auxin paste cannot be due to an obstruction by the paste or diversion towards it of any of the substances necessary for growth, which were coming from the roots and cotyledons: for the inhibited farther halves of the young split internodes were in the line between the paste on the one hand and the roots and cotyledon below them on the other hand (the cotyledons were not exhausted at the end of the experiment).

But the results could be interpreted on the theory which the writer previously (1937, p. 294) set out and supported more fully for correlative inhibition. On this theory the inhibiting effect is indirect and due to a secondary influence of some kind (possibly another hormone) which can travel easily in either morphological direction. In all the parts of the stem which the auxin, travelling mostly in the morphologically downward direction, reaches in plenty, it counteracts

and overcomes the inhibiting influence: but in those parts which no auxin reaches, or not much, because they are separated from the source of auxin by a zone of tissue in which it would have to travel morphologically upwards, in them the inhibiting influence produces its effect. However it needs also to be considered whether the upward inhibition by auxin paste may depend on transport of the auxin with the transpiration stream, even though the correlative inhibition of buds on a side-shoot does not depend on it (Snow, 1937, p. 286).

It is further of interest to compare the results of the last experiment with those of the following simpler experiment (Exp. 6, Figs. 2*a*, *b*), in which a young internode of a pea shoot was carefully split longitudinally in the plane of the leaves as before, and the cut was continued a little way (about 1 cm.) down into the internode below. Then one of the half-stems so formed was cut through transversely, at the level of the base of the split, so that it formed a downward-pointing strip attached at the top only. This half will be called the "free" half, and the other the "non-free" half. In previous experiments with *Vicia Faba* the writer (1932, p. 92) had found that both such half-stems elongate rapidly. The shoots were defoliated and decapitated close beneath the apex, and the cut surfaces were washed and (except where paste was to be applied) vaselined as before: the plants were grown and kept in similar conditions. A large ring of hetero-auxin paste, 1 in 600, was now applied to one half of the upper part of the internode next below the young split internode—either (*a*) to the "non-free" half (Fig. 2*a*) or (*b*) to the "free" half (Fig. 2*b*). Controls were similarly operated on, but received no paste. The mean lengths of the halves of the young split internodes after 11 days, and of the higher internodes which were too small to measure at the start, are given in Table VI.

In Table VI it can be seen, by comparing group "*a*" with the controls, that when the auxin paste was at the base of the "non-free" half, both halves of the young split internode were strongly inhibited. This is what would be expected in view of the results of Exp. 5, but it is worth noting that the strength of inhibition was only very slightly greater than in Exp. 5, in which each half-internode was connected directly with the roots and cotyledon below it. Indeed the half directly above the paste in this group was inhibited only just about as strongly as the corresponding half in Exp. 5, while the other half (the "free" half) was inhibited only slightly more strongly than the corresponding half in Exp. 5. The next higher internode, however,

TABLE VI. *Exp.* 6 (*see Figs. 2 a, b*)

	At start, mean length in mm. of split internodes	After 11 days: Split internodes: Non-free half	After 11 days: Split internodes: Free half	After 11 days: Next younger internodes	After 11 days: Next younger internodes again
Experiments "*a*", 4 plants	6·75 (extremes 5·5 and 8·5)	18·25 $\sigma_m = 2{\cdot}16$	15·0 $\sigma_m = 1{\cdot}28$	3	Not elongating
Experiments "*b*", 6 plants	7·17 (extremes 5·0 and 8·5)	37·2 $\sigma_m = 3{\cdot}39$	25·3 $\sigma_m = 2{\cdot}60$	57·3	31·7 (4 plants only)
Controls, 6 plants	6·78 (extremes 5·5 and 9·0)	36·1 $\sigma_m = 3{\cdot}00$	30·7 $\sigma_m = 2{\cdot}71$	66·1	45·0 (4 plants only)

was inhibited distinctly more strongly in this group than in Exp. 5: indeed it was completely arrested at a length of only from 2 to 4 mm.

But the plants of group "*b*", with the paste at the base of the "free" half, showed a quite unexpected result when compared with the controls. For they showed no trace of inhibition, neither in the halves of the young split internode nor in the still younger internodes above, of which two had elongated during the experiment. If this result is compared with that of Exp. 5, it is clear that the upward inhibiting effect of auxin paste is enormously diminished when the zone of stem to which the paste is applied is deprived of its direct connexion with roots and cotyledon below, by being cut through just below the paste. To explain this fact, further experiments are needed.

However it should not be concluded that auxin paste applied in this way to the free base of a cut stem or half-stem produces *absolutely* no inhibiting effect above: for a more sensitive test, perhaps one involving the outgrowth of a small bud, might reveal some inhibiting effect. Indeed Le Fanu (1936), after applying auxin in gelatine to the morphological base of an inverted cutting of a pea shoot found that the bud at the node above was partially inhibited.

It was again noticeable in Exps. 5 and 6 that the greater part of the inhibited zones above the auxin paste did not swell. The zones to which the paste was applied swelled considerably, and the swellings extended downwards and also a little way upwards into the lower part of the half-internode directly above the paste. But they did not extend so far as half-way up this half internode, nor of course into the other half internode or higher internodes. In this respect, as already pointed out, the upward inhibiting effect of auxin paste differs

conspicuously from the much slighter inhibiting effect which is sometimes exerted by a strong paste applied from above: for the latter effect seems always to be accompanied by swelling of the inhibited parts. An interesting point noticed was that in the plants of group "*b*" of Exp. 6, in which the paste was applied to the lower ends of the "free" half-stems and the parts above were not inhibited, the swellings were not much smaller, if at all, than in the plants of Exp. 5 and of group "*a*" of Exp. 6, in which the parts above the paste were strongly inhibited.

SUMMARY

1. Rings of hetero-auxin paste placed round young internodes of pea shoots strongly inhibit, or completely arrest, the elongation of the younger internodes above, as was discovered by Le Fanu (1936). It is shown that, contrary to what Nagao (1937) expects, decapitation does not in any way interfere with this effect. The inhibited internodes do not grow in thickness either, except for a short zone immediately above the paste.

2. When the same hetero-auxin pastes, 1 in 2000 and 1 in 600, were applied to the tops of similarly decapitated pea shoots, the weaker paste did not affect the elongation of the young internodes below, nor did the stronger paste, except that it did slightly inhibit the elongation of the very young internodes which were too small to measure at the beginning of the experiment. Both pastes, when applied on top, made all the young internodes grow in thickness very strongly.

3. It is concluded, in agreement with Le Fanu (1936), that the upward inhibiting effect of hetero-auxin paste applied below is quite different from any effect exerted by the same paste when applied above.

4. An experiment is reported which shows that the inhibiting effect of the paste upon the parts morphologically above it does not depend upon the morphological direction in which any substance or influence coming from the paste may be travelling in those parts; nor does it depend on any obstruction or diversion of substances needed for growth. In these respects it is similar to correlative inhibition.

5. Hetero-auxin paste, when applied to the free lower end of a downward-pointing strip of young stem attached at the top only, does not inhibit the parts above it, or only very slightly, if at all.

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REVIEWS

Introduction to Plant Pathology. By FREDERICK DEFOREST HEALD. Pp. ix + 579, with 200 figures. McGraw-Hill Publishing Co., Ltd. London. 1937. Price 24s.

This is, preface notwithstanding, essentially a "boil-down" of the author's *Manual of Plant Diseases* first published in 1926 and issued in a larger and, to a great extent, re-written second edition in 1933, and considerable portions of the type of this appear to have been used. The number of fungus diseases treated in detail has been drastically reduced and new chapters of more popular appeal have been introduced, e.g. chapters on the relations of fungi and of plant diseases to human affairs and the dissemination of plant diseases (three chapters in all) as well as on plant disease control (four chapters) and plant pathology methods (three chapters). The order of presentation has also been altered and diseases caused by bacteria, fungi, viruses, etc., are now dealt with before the non-parasitic troubles. The method of teaching is by types and in consequence of the curtailment this is not so useful as a work of reference as the author's *Manual* or Brooks's *Plant Diseases*.

The chapters on the relations of fungi, bacteria and plant diseases to human affairs are somewhat encyclopaedic but they possess a certain charm suggestive of the Rolfes' *Romance of the Fungus World*. These form a pleasant introduction to the subject and are followed first by a chapter on the dissemination of plant diseases and then by the text dealing with diseases caused by parasites, viz. fungi, bacteria and parasitic flowering plants. For the most part the subject-matter is acceptable to the English teacher though a few of the diseases are exclusively American. The chapter on diseases caused by bacteria is perhaps the weakest from this point of view, for the diseases dealt with in detail are Fire Blight, of which the European records are entirely unsubstantiated, Crown Gall, the chief importance of which in Europe lies in the American market for certain nursery produce, and Black Rot of Crucifers which though of wide distribution is not here of particular importance. Diseases of plants caused by eelworms are, in America, studied by botanists, hence the inclusion of a chapter on diseases caused by nematodes. Owing to the similarity of certain of these diseases to attacks by fungi and bacteria some knowledge of them is a useful extra in the training of a plant pathologist, and the necessary information is well provided here.

The treatment of diseases caused by viruses suffers from the disadvantage which has been mentioned in connexion with the chapter on diseases caused by bacteria. The first of the two chapters is a useful and up-to-date introduction to the study of viruses, but the second, apart from a somewhat slight treatment of virus diseases of the potato with emphasis on Leaf Roll, deals with diseases of almost exclusively American importance, viz. five local virus diseases of the peach in America, curly top of beet and aster yellows. Excellent as is the treatment of the last mentioned two diseases, this chapter would need a considerable supplement to be of real use to English students.

The chapters on viruses are followed by those on non-parasitic diseases on lines similar to that used in the earlier volumes, and the ground seems to be well covered. A section on plant disease control and a very short one on methods of study complete the work.

The volume is well printed and bound, and the illustrations are, on the whole, commendable. Fig. 33 shows, correctly, the conidiophores of *Phytophthora infestans* as positively geotropic but the same treatment has not been accorded to those of *Plasmopara viticola*, fig. 40, in spite of the fact that their mode of growth is evident from a consideration of fig. 39.

Misprints are few and far between, but the misspelling of Craigie on p. 10, of Arran on p. 347, and the ridiculous use of "sighted" for "cited" on p. 436, have all been carried over, presumably with type from the second edition of the manual. The misspelling of amadou on p. 27, and of Tingid on p. 318, seem likely to escape notice should another edition be required.

The recent conversion of the author to the use of the ugly word "virous" is to be deplored. English is after all, at heart, a Teutonic language, and the convenience of building up strings of nouns is not one to be lightly thrown away. The author takes the fullest advantage of it in his use of the phrase "plant pathology methods" as the heading of Section VI. Monstrosities such as "pathological laboratory" and the difficulty of "rationalizing" "plant pathology" or better still "Independence Day" may be cited as evidence in favour of the retention of virus and fungus as adjectives.

With the reservation regarding the extra reading on diseases caused by bacteria and viruses the work may be commended to students in this country until something as good in a similar style appears here. The price is, however, somewhat high for the average student, though, bearing in mind the binding, it is not unreasonable. Judged as an American work for American readers the work seems worthy of very great praise indeed. ALEX. SMITH

Recent Advances in Cytology. By Dr C. D. DARLINGTON. 2nd end. Pp. 671, with 16 plates, 160 text-figures and diagrams and 78 tables. J. & A. Churchill. 1937. Price 21s. net.

In the cell, and especially the nucleus, are integrated the system of growth, reproduction, heredity and variation that makes up any organism. Further, the cell is related in its structure and behaviour to events in its ancestors. The uniformity of cell behaviour, and particularly of nuclear mechanisms, renders it possible to trace by induction the manner in which the diversities have arisen. In the first edition of this book, Dr Darlington devoted the last chapter to an attempt to show that cell processes are themselves the products of evolution of the genetic system they maintain. This point of view, much strengthened by many more cases of genotypic control of cell processes, has now pervaded his whole treatment of cytology, especially in its nuclear aspects.

Meiosis, an indispensable adjunct of sexual reproduction, is shown to be related to mitosis as a precocious variant in which the external habiliments of nuclear division are present before the internal division of the chromosome. At the earliest prophase of mitosis the chromosomes are double; at leptotene they are single. Dr Darlington deduces that homologous parts of chromosomes attract one another in pairs at certain stages in the nuclear cycle. At mitosis, this attraction is satisfied internally by the two chromatids of one chromosome; at meiosis externally by pairing of homologous parts. This conclusion has been questioned, largely on the basis of observations of optical appearances suggesting double, or even quadruple, structure in anaphase chromosomes at somatic mitosis. But the division of the chromosomes during the resting stage—not earlier—seems proved beyond all question in another way. Riley and Mather have shown that the chromosomes change, during the resting stage preceding the first pollen-grain mitosis, from a single to a double structure with respect to their reaction to X-rays.

A second important deduction made is that metaphase pairing at meiosis is conditioned normally by the presence of chiasmata, which are the result of cytological, and therefore genetical, crossing-over. The absence of crossing-over in the *Drosophila* male, which has a regular meiosis, is related to the absence of chiasmata in the autosomal bivalents. Instead, these are held together at metaphase by a special attraction, an exaggeration of the property of somatic chromosome pairing so characteristic of this and other Diptera.

Meiosis has therefore arisen from mitosis and, with its two properties of pairing and of crossing-over, made possible the further complexities of sexual reproduction. Diploidy has variously given rise to polyploidy or to structural hybridity; and these, through their conditional properties of conservation of heterozygosity, have given rise respectively to allopolyploid and to complex heterozygote types of genetic organization. The latter combines in itself the advantages of clonal and of sexual reproduction. Sexual differentiation is a special case of it, which finally, as a breakdown in the genetic system, has given rise to apomixis.

In the final chapter, an attempt is made to co-ordinate our knowledge of cell mechanics. Three interrelated aspects are treated, namely movements of chromosomes (external mechanics), changes in shape of chromosomes (internal mechanics) and movements related to crossing-over and structural change (ultra-mechanics). The conventions of Newtonian mechanics are adopted in explaining cell mechanics in terms of forces of attraction, of repulsion or of torsion.

Changes in the shape of chromosomes are conditioned by a spiralization cycle, dependent upon a torsion (molecular spiral) within the chromosome thread. There is a lag in uncoiling of the chromosome induced by a relatively rigid medium. Specific attractions of the parts of chromatids for one another and a predetermined cleavage plane are added factors responsible for relational coiling of chromatids. Crossing-over is explained as a relief of the extra strain introduced when paired and relationally coiled chromosomes divide at the end of pachytene.

The external movements of chromosomes depend upon the specific attractions between parts of chromatids and the repulsions situated generally on the chromosome surface, on the centromeres and on the centrosomes or other cell organs representing them functionally.

All these movements, co-ordinated in time, are related in cause which must be expressed in the colloidal properties of the protoplasm. The cycles of change in the surface changes—actually double electrical layers—on the different cell organs which are amphoteric electrolytes are related to cyclical changes in the *p*H of the medium. The orderly sequence of changes in the cycle of chromosome, nuclear and cell division is therefore dependent upon the maintenance of a proper balance between the constituent events.

The whole book is a sequence of close argument leading by inferences from a comparison of the simpler, well-established, facts of cytology to an analysis of the more difficult and controversial phases. The validity of the deductive method employed is shown by the frequency with which successful predictions follow from its application and by the discovery of previously unknown principles following a false prediction. The book not only marks an advance in cytological knowledge, but also an advance in biological method.

Like the first edition, it is well illustrated by explanatory diagrams and by photomicrographs; it is completed by a glossary of current cytological terms and an extensive bibliography.

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TAXONOMY AND RELATIONSHIP IN THE GERANIALES IN THE LIGHT OF THEIR CYTOLOGY

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PART II

Erodium

This genus differs from *Geranium* in having five fertile and five sterile stamens instead of ten fertile ones, in the method of shedding of the fruit (the ovary wall coming off with the seed instead of remaining attached), and in the tendency to pinnate rather than palmate division of the foliage.

The genus is divided by Knuth (1912) into two sections: I, *Plumosa* and II, *Barbata*. The former contains five species, of which one has been available. The latter is divided into ten subsections, of which representatives of eight have been examined.

This classification is on the whole satisfactory. *E. ciconium* has, however, been transferred from the subsect. *Absinthoidea*, from which it differs in its annual habit and slaty coloured hermaphrodite flowers, to the subsect. *Gruina*, with which it agrees in all respects except in its rather more pinnate leaves.

The behaviour at meiosis of the species of this genus has not proved easy to observe, owing to the small size both of the anthers and chromosomes. It presents, however, no important differences from that of *Geranium* and will not be considered further.

The somatic chromosomes also present no striking features. They are small in size and very uniform in most species. No differences comparable with those found in *Geranium* exist in any species examined. The meiotic chromosomes also are uniform in size throughout the genus.

As the genus is fairly uniform cytologically it will be considered as a whole, Knuth's classification being followed.

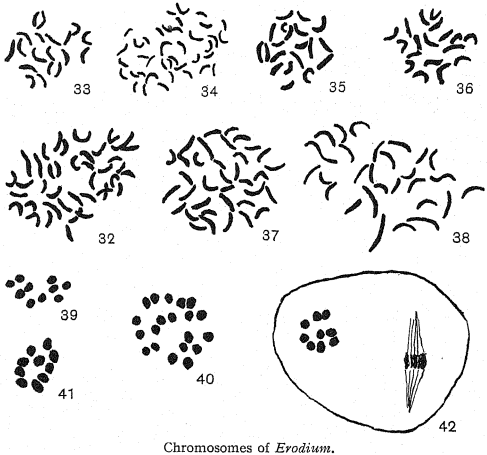
	n	2*n*	Locality
Sect. I. **Plumosa** Boiss.			
E. glaucophyllum (L.) L'Hér.	.	20	
Sect. II. **Barbata** Boiss.			
Subsect. *Pelargoniiflora* Brumh.	.	.	
E. hymenodes L'Hér.	10	20	
Subsect. *Malacoidea* Willk. & Lange	.	.	
E. chium (Burm f.) Willd.	20	.	
E. malacoides (L.) Willd.	.	40	
Subsect. *Chamaedryoidea* Brumh.	.	.	
E. corsicum Leman	10	.	
E. chamaedryoides L'Hér. f. *roseum*	.	20	
Subsect. *Gruina* Willk. & Lange	.	.	
E. gruinum (L.) L'Hér.	.	40	
E. ciconium (L.) Ait.	.	20	
Subsect. *Absinthoidea* Brumh.	.	.	
E. Guiccardii Heldr. ♂	.	28 ?	
E. chrysanthum L'Hér. ♂ (Fig. 37)	.	36	
E. chrysanthum L'Hér. ♀	.	36	
E. absinthoides var. *amanum* Brumh. ♂ (Fig. 42)	9	18	
E. absinthoides var. *amanum* Brumh. ♀ (Fig. 36)	.	18	
E. chrysanthum × ? ♂	.	32 ?	
Subsect. *Petraea* Brumh.	.	.	
E. petraeum (Gouan) Willd.	10	20	
E. cheilanthifolium Boiss. (Figs. 32, 40)	20	40	
Subsect. *Cicutaria* Willk. & Lange	.	.	
E. cicutarium (L.) L'Hér. (Figs. 33, 34)	.	20	Blakeney Point, Norfolk
	.	20	Merthyr Mawr, Glam.
	.	40	Mendip Hills, Somerset. (Near Wells)
	.	40	Aberdeen
	.	40	Newcastle, Co. Down
	.	40	Borth, Cardigan
	.	40	Freckenham, Suffolk
E. cicutarium (L.) L'Hér. (Figs. 33, 34) var. *pimpinellifolium* Cav.	.	40	
E. moschatum (L.) L'Hér. (Figs. 35, 41)	10	20	
Subsect. *Romana* Brumh.	.	.	
E. Manescavii Guss.	.	40	

This is a fairly representative selection of the genus. The genus is uniform cytologically, the only striking variation being found in the subsect. *Absinthoidea*, which differs in basic number from the rest of the genus. Tetraploids occur in several of the subsections, but no higher polyploids have been found.

E. cicutarium is of particular interest. It is an aggregate species, split into several segregates which are not yet properly understood. For this reason no attempt has been made in the above list to give the segregate species. All the inland forms examined, and some of the maritime ones (e.g. from Newcastle) are tetraploid, but two diploids, both maritime, occur. These present certain morphological differences, but great difficulty has been encountered in growing these forms satisfactorily, and therefore of determining the segregates.

The other feature of interest in *Erodium* is presented by the subsect. *Absinthoidea*. This subsection has varying chromosome numbers. It differs morphologically from the rest of the genus in having dioecious flowers, a feature not mentioned by Knuth.

No sex chromosomes have been detected in either of the species of which female plants have been available. More variation in chromo-



Chromosomes of *Erodium*.

Fig. 32. *E. cheilanthifolium*. Mitotic metaphase.
Fig. 33. *E. cicutarium* (from Blakeney Point, Norfolk). Mitotic metaphase.
Fig. 34. *E. cicutarium* var. *pimpinellifolium*. Mitotic metaphase.
Fig. 35. *E. moschatum*. Mitotic metaphase.
Fig. 36. *E. absinthoides* var. *amanum* ♀. Mitotic metaphase.
Fig. 37. *E. chrysanthum* ♂. Mitotic metaphase.
Fig. 38. *E. Guiccardii* ♂; meiotic division.
Fig. 39. *E. hymenodes* first division metaphase.
Fig. 40. *E. cheilanthifolium* first division metaphase.
Fig. 41. *E. moschatum* second division metaphase.
Fig. 42. *E. absinthoides* var. *amanum* male, second division metaphase.

some size occurs within the species in this section than is usual in the genus. This is particularly marked in *E. Guiccardii*.

The number $2n = 32$ occurs in a plant with cream-coloured flowers which is not identifiable with any known species, and is probably a

hybrid between *E. chrysanthum* and some other species, possibly *E. Guiccardii*. No meiotic plates of this plant have, as yet, been examined.

Examination of more species of this section is desirable before its cytology is clear. There seems to be a possibility of a basic number $n=9$, from which the other numbers have arisen. The size variation in *E. Guiccardii* indicates that fragmentation may have occurred in this species, whereas in the species with $n=9$ and 18 the chromosomes are more uniform.

Erodium is much more restricted than *Geranium* in its geographical distribution. Of the sixty species of the genus, only seven are not found in either Europe or the Mediterranean region. Of these one is Central Asiatic (four other species also occur in this region), one East Asiatic, one South African, two western North American, one South American and one Australasian. There is thus one centre of distribution, the Mediterranean region, where forty-six species occur, with an extension into Central Europe, in contrast to the several centres of distribution in *Geranium*. This points to a more recent origin of *Erodium* and this is confirmed by the morphological features, particularly the sterility of five of the stamens and the tendency to zygomorphy.

Cytologically the genus contrasts with *Geranium* in its much greater uniformity and in the more widespread polyploidy. Both these features agree with the idea of a more recent origin. It compares cytologically with such genera as *Papaver* and *Trifolium*, both of which it resembles in having a basic number running through the genus with one exceptional section.

The chromosomes number $n=10$ in the genus probably originated from some primitive Geranioid type with $n=7$ by reduplication of chromosomes, in the same manner as has been suggested for the sections of *Geranium*, but in the absence of definite evidence of secondary association the exact method remains uncertain. The number 9 in subsection *Absinthoidea* must be regarded as derived from this number by loss of a chromosome, as the subsection is morphologically advanced in being dioecious and geographically restricted, but not otherwise greatly different morphologically from the rest of the genus. This indicates that a homology probably exists, or has existed, among some of the ten chromosomes of the other members of the genus, for loss to take place without unbalance. The origin of the other numbers in this section is obscure. The variation in length indicates a possibility of fragmentation of an $n=9$ species. There is also a possibility

that hybridization may have played a part, as polyploidy exists in the subsection and hybrids certainly occur.

Hybridity in this genus occurs with some frequency and certain hybrids have been artificially produced by the writer. These died without flowering and their cytological behaviour is therefore unknown.

The occurrence of hybridity in the genus affords additional evidence for its comparatively recent origin. It suggests further that hybridity may have played a part in the origin of the tetraploid species.

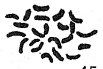
Monsonia

	n	*2n*
M. senegalensis (Fig. 43)	.	24

Of the twenty-nine species of the genus, only one has been available, and of this only somatic preparations. The chromosomes are small for the family (Fig. 62).

43

44



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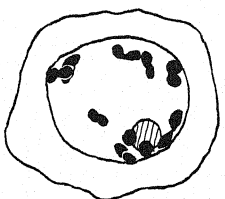

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Chromosomes of *Monsonia* and *Pelargonium*

Fig. 43. *Monsonia senegalensis* mitotic metaphase.
Fig. 44. *Pelargonium hybridum* mitotic metaphase.
Fig. 45. *P. glaucifolium* mitotic metaphase.
Fig. 46. *P. hybridum* "Madame Kovelesky" diakinesis.

Morphologically the genus is distinct in having fifteen stamens. All the species are African, though one extends into Asia. It is presumably fairly recent, but speculations on phylogeny are valueless until more species have been studied.

Pelargonium

	n	2*n*	
Sect. **Hoarea**			
P. punctatum (Andr.) Willd.	.	22	Warburg
Sect. **Polyactium**			
P. ×*glaucifolium* Sweet (Fig. 45)	.	22	Warburg
Sect. **Jenkinsonia**			
P. Endlicherianum Fenzl.	.	36	Warburg
Sect. **Dibrachya** (Sweet) Haw.			
P. peltatum (L.) Ait.	.	36	Takagi
P. lateripes L'Hér.	9	.	Warburg
Sect. **Ciconium** (Sweet) Haw.			
P. inquinans (L.) Ait.	.	18	Takagi
P. ×*hybridum* Ait. Several forms (Fig. 44)	.	18	Takagi & Warburg
P. Madame Kovelesky (Fig. 46)	9	.	Warburg
P. zonale (L.) Ait.	18	36	Takagi
Sect. **Cortusina** DC.			
P. odoratissimum (L.) Ait.	.	16	Takagi
Sect. **Pelargium** (DC) Harvey			
P. tomentosum Jacq.	.	45	Takagi
P. quercifolium (L. fil.) Ait.	.	45	Takagi
P. "×*domesticum*" class	.	45	Takagi
P. radula (Cav.) L'Hér.	.	81	Takagi
P. denticulatum Jacq.	.	90	Takagi
P. graveolens (Jacq.) L.Hér.	.	90	Takagi
P. glutinosum L'Hér.	.	90	Takagi

This enormous genus of about 230 species has, for several reasons, not been examined in any detail. The chromosomes of several species have been counted by Takagi (1928). The genus has hybridized freely in gardens, and thus makes garden plants difficult to identify and uncertain as to origin. Finally, the genus is morphologically advanced in its zygomorphic flowers and spurred petals, and geographically nearly confined to South Africa. It was thought therefore that its investigation was best left until the other genera of the family were fairly well known. The genus will therefore only be dealt with briefly.

The somatic chromosomes are small and, in the species examined both by Takagi and by the present writer, similar to those of the other genera. The reduction division has been studied in *P.* "Madame Kovelesky", a hybrid of the class called by Takagi *P. hortorum*. This class of hybrids is generally supposed to have originated from the

hybrid *P. zonale* × *P. inquinans* (*P. hybridum* Ait.). It seems probable therefore that Takagi's *P. zonale*, which is stated to be a garden form, is either a tetraploid form of the diploid species *P. zonale*, or itself originated by hybridization. The question must be left open until plants of true wild *P. zonale* have been examined.

The reduction division in this hybrid is perfectly normal, the haploid number being 9. No trace of non-conjunction or other irregularities occurs. The chromosomes of the parents must therefore be considered as perfectly homologous. In essentials the reduction division is similar to that of *Geranium cinereum*, but no ring-shaped bivalents have been observed at diakinesis. At metaphase the chiasmata are mostly completely terminalized but occasionally interstitial chiasmata have been observed. It therefore agrees with *Geranium* in this respect.

Of the fifteen sections into which Knuth divides the genus, chromosome numbers are known for seven. Of these sections four have the basic number 9; of the other three, in each of which only one species has been counted, two have $n=11$ and one $n=8$. It is impossible to say at present whether polyploids exist on these as basic numbers.

The section *Pelargium* is of interest; the occurrence of an enneaploid suggests that extensive hybridization has gone one in this section. It is probable that such plants are garden hybrids and do not rightly belong to the sections to which Takagi (1928) attributes them.

As stated above, the genus is morphologically advanced as compared with *Geranium*, which agrees with the idea of the former existence of a member of the family with $n=7$, the numbers 8, 9, 11 of *Pelargonium* being derived from it by polysomy. Hybridity, which is known to occur rather commonly in *Pelargonium*, has probably played a part in the origin of the polyploids in the genus; this suggests that hybridity may also have occurred in *Geranium* in the past.

Takagi's work shows two possible ways in which polyploidy may have occurred. The first is by somatic doubling, which he found in the roots of *Pelargonium zonale*. The second is by non-conjunction in pollen grains, giving rise to diads instead of tetrads. This occurred in cold weather.

OXALIDACEAE

This family is monographed by Knuth in the *Pflanzenreich* (1930). Of the seven genera, six are small and have not been available. The remaining genus, *Oxalis*, has nearly 800 species, of which the chromo-

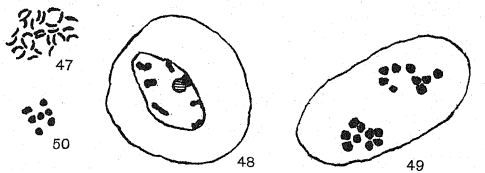
some numbers are known for about twenty-five. The genus is divided by Knuth into thirty-seven sections. Some numbers are known in fifteen of these.

Oxalis

	n	*2n*	
Sect. **Thamnoxys** (Planch.) Prog.			
O. rhombifolia Jacq.	.	> 80	Heitz
Sect. **Heterophyllum** Prog.			
O. bupleurifolia A. St Hil.	.	10	Heitz
Sect. **Ortgieseae** R. Knuth			
O. Ortgiesii Regel (Figs. 48, 49)	7	.	Warburg
O. Ortgiesii Regel	.	14	Heitz
O. tuberosa Molina	.	14	Heitz
Sect. **Laxae** (Reiche) R. Knuth			
O. valdiviensis Barn. (Fig. 50)	9	.	Warburg
Sect. **Roseae** (Reiche) R. Knuth			
O. rosea Jacq.	.	14	Heitz
Sect. **Articulatae** R. Knuth			
O. articulata Savign.	.	14	Heitz
O. rubra A. St Hil.	.	42	Heitz
Sect. **Acetosellae** (Reiche) R. Knuth			
O. acetosella L.	.	22–24	Heitz
Sect. **Palmatifoliae** Reiche			
O. adenophylla Gill.	.	28	Heitz
Sect. **Jonoxalis** (Small) R. Knuth			
O. grandifolia DC	.	14	Heitz
O. vespertilionis Zucc.	.	14	Heitz
O. Drummondii A. Gray	.	14	Heitz
O. violacea L.	.	28	Heitz
Sect. **Polyoxalis** R. Knuth			
O. Deppei Lodd.	.	14	Heitz
O. lasiandra Zucc.	.	28	Heitz
Sect. **Cernuae** R. Knuth			
O. purpurata Jacq.	.	28	Heitz
O. purpurata var. *Bowiei* (Lindl.) Sond.	.	28	Heitz
O. caprina L.	.	20	Heitz
Sect. **Tripartitae** R. Knuth			
O. versicolor L.	.	14	Heitz
O. incarnata L.	.	14	Heitz
O. Smithiana Eckl. and Zeyh.	.	14	Heitz
O. Tenuifolia Jacq.	.	28	Heitz
O. truncatula Jacq.	.	42	Heitz
Sect. **Pteropodae** DC.			
O. asinina Jacq. (Fig. 47)	.	28	Heitz & Warburg

	n	*2n*	
Sect. **Multifoliatae** R. Knuth			
O. peniaphylla Sims	.	28	Heitz
Sect. **Sessilifoliatae** DC.			
O. hirta L. var. *rubella* (Jacq.) R. Knuth	.	28	Heitz

The chromosomes of the genus, both somatic and meiotic, are small. Size variations are not great, as far as is known. The somatic chromosomes show no satellites or marked constrictions, and are very similar in appearance to those of the Geraniaceae. The reduction division is also similar to that of the latter family. Diakinesis is difficult to observe. As far as has been made out in the species



Chromosomes of *Oxalis*

Fig. 47. *O. asinina* mitotic metaphase.
Fig. 48. *O. Ortgiesii* diakinesis.
Fig. 49. *O. Ortgiesii* second division metaphase.
Fig. 50. *O. valdiviensis* second division metaphase.

examined, *O. Ortgiesii*, the chiasmata are all terminalized at this stage (Fig. 69), as is usual in the Geraniaceae. At metaphase they are certainly completely teminalized both in this species and in *O. valdiviensis*.

The present writer has not attempted many counts for the genus, as most of the obtainable species have been counted by Heitz (1927).

From the list given above it will be seen that in ten of the fifteen sections the basic number is 7. It occurs also in one of the remaining sections. For the remaining four sections the numbers are various, and rest on only one species in each case, so that it is impossible to tell if polyploidy on numbers other than 7 occur here, or whether aneuploidy or a constant number prevails in these sections.

Geographically the genus is mainly South American, with a good many species extending north to Mexico. Six allied sections occur in Africa (these are sections 32–37), and one section, Acetosellae, extends to Europe, Asia, Australia and New Zealand. It is noteworthy

that the African group has 7 as its basic number like the South American. Of the species with aberrant numbers, *O. rhombifolia* in *Thamnoxys* and *O. bupleurifolia* in *Heterophyllum*, belong to sections which are rather distinct morphologically (the former in the petioluled terminal leaflet and the leaflets not obcordate, the latter in the absence of leaflets) but the remaining aneuploid species show no marked morphological characters.

It is almost certain that 7 is the basic number in the genus, and that the other numbers have been derived from this at various periods. Until more is known of the behaviour within the sections the genus cannot conveniently be compared with other genera, but it shows a certain resemblance to the Geraniaceous genera in the presence of one number on which polyploidy occurs, together with scattered aneuploidy.

A certain variation of chromosome size exists in the genus, the somatic chromosomes of *O. bupleurifolia* according to Heitz's figures being considerably larger than those of most species.

TROPAEOLACEAE

Of this family little can be said at present. According to Buchenau's (1902) monograph in *Das Pflanzenreich* it consists of a single genus *Tropaeolum* of about fifty species, but Farenholtz (1931), in his account in *Die Natürlichen Pflanzenfamilien*, considers *Magallana* distinct.

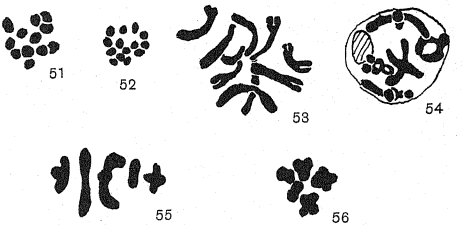
Tropaeolum

	n	*2n*	
T. peltophorum Benth.	.	*c.* 28	Heitz
T. minus L. (Fig. 52)	14	.	Warburg
	.	27–28	Heitz
T. majus L.	14	.	Sugiura
		27–28	Heitz
T. peregrinum L. (Fig. 51)	12	.	Sugiura & Warburg
		26–30	Heitz

Counts are known only for four species. Of these three, *T. minus*, *T. majus* and *T. peltophorum*, are closely allied. These all, apparently (the two first certainly), have $n=14$. The remaining species, *T. peregrinum*, has $n=12$. Heitz's count of $2n=26$–30 for this species is unintelligible. Sugiura (1928) in his careful investigation of the pollen of this species gives $n=12$ and my counts confirm this. Heitz was perhaps dealing with some other species, though the species is almost unmistakeable, or the count may be an error, or two strains may exist.

On the evidence available it is therefore impossible to say what is the primitive number in this genus, though the occurrence of fourteen is suggestive when considered in relation to *Oxalis* and *Geranium*.

The reduction divisions of both *Tropaeolum majus* and *T. peregrinum* have been studied by Sugiura (1925, 1928). My observations confirm his in the main, but the question of whether the number of ring-shaped bivalents at diakinesis is really constant requires further study. For the purpose of this paper it is sufficient to note that the



Chromosomes of *Tropaeolum* and *Limnanthes*

Fig. 51. *Tropaeolum peregrinum* first division metaphase.
Fig. 52. *T. minus* second division metaphase.
Fig. 53. *Limnanthes alba* mitotic metaphase.
Fig. 54. *L. Douglasii* diakinesis.
Fig. 55. *L. Douglasii* first division metaphase spindle view (chromosomes drawn separately).
Fig. 56. *L. Douglasii* first division metaphase, polar view.

chiasmata are completely terminalized by diakinesis, and that the bivalents at this stage are either ring- or dumbbell-shaped.

Geographically the family is confined to South America.

LIMNANTHACEAE

Limnanthes

	n	*2n*	
L. alba Hartw. (Fig. 53)	5	10	Warburg
	.	10	Fries (1936)
L. Douglasii R.Br. (Figs. 54–56)	5	.	Stenar (1925)
	5	.	Warburg
	.	10	Fries

This small family, which is entirely North American, consists of the two genera *Limnanthes*, with three or four species, and *Floerkea* with one species. Only the former, of which two species, *Limnanthes alba* and *L. Douglasii*, have been examined, has been available. These both have $n=5$.

The somatic chromosomes are larger than in most of the plants studied, being comparable in size with those of *Geranium polyanthes*. The constrictions are median or submedian in all the pairs (Fig. 53). I have only observed them in *Limnanthes alba*, but Fries (1936) states that those of *L. Douglasii* are identical.

The embryology and cytology of *L. Douglasii* have been studied by Stenar (1925), but mainly in connexion with the ovule. He figures and describes the second meiotic division, but ignores the first division.

The first meiotic division of *L. Douglasii* is strikingly different from that of any of the plants previously described in this paper. At diakinesis the chromosomes are larger than in any of the other plants studied. The chiasmata at diakinesis (Fig. 54) in *Limnanthes* are mainly interstitial. The total number of chiasmata is about seven or eight, and the terminalization coefficient between 0·3 and 0·4, instead of the practically complete terminalization in all the previous plants described. Various configurations have been observed, the total number of chiasmata varying from one to three in each bivalent, all of which have been observed with any possible number terminal.

At metaphase the terminalization coefficient reaches about 0·6. Of the bivalents at this stage two or three have terminal spindle attachment (usually one of each on each spindle), and either terminal or subterminal chiasmata. The others with median attachment are more difficult to observe, the configurations existing at diakinesis seem also to exist here but with a greater degree of terminalization (Figs. 55, 56).

The chromosomes in the second division are visible as **V**-shaped structures at metaphase.

Meiosis has not been closely studied in *L. alba* but is believed to be similar.

There are thus considerable cytological differences between *Limnanthes* and all the plants previously described in relation to number, morphology and behaviour during reduction division. The taxonomic significance of this will be discussed later.

BALSAMINACEAE

Impatiens

	n	*2n*	
Subgen. *CAULIMPATIENS*			
Sect. **Salpingochilon**			
I. Roylei Walp. (Figs. 58, 62)	10	.	Warburg
	(9)	.	Wulff

	n	*2n*	
Sect. **Microcentron**			
I. Balsamina L. (Fig. 59)	7	14	Several authors
	7	.	Warburg
I. rosea Lindl.	7	.	Wulff
Sect. **Macrocentron**			
I. capensis Thunb.	7	.	Wulff
I. firmula Bak.	7	.	Wulff
I. Sultani Hook. f. (Figs. 57, 60 and 61)	8	16	Warburg
	8	.	Wulff
	(7)	.	Ottley
Sect. **Brachycentron**			
I. Noli-tangere L.	.	20	Winge
I. parviflora DC	.	20	Heitz
	10	.	Schürhoff
	12	.	Wulff
I. biflora Walt.	10	.	Wulff
I. aurea Muhl. (=*pallida* Nutt.)	12?	.	Raitt
	10	.	Wulff
Sect. **Brevicornes**			
I. amphorata Edgw.	7	.	Warburg
	7	.	Wulff
Sect. **Longicornes**			
I. Holstii Engl. & Warb.	.	16	Warburg
	8	.	Heitz

Counts in brackets are considered to be errors.

Hydrocera

H. triflora (L.) W. & Arn.	.	16	Heitz
	8	.	Schürhoff

The family consists of two genera only, as given above. *Hydrocera* contains only one species.

Impatiens, according to O. Warburg and Reiche (1896), consists of about 220 species (numerous species have been described since), of which 150 occur in tropical Asia, forty-six in tropical Africa, and eight in temperate Europe, Asia and North America. They divide the genus into two subgenera and eighteen sections.

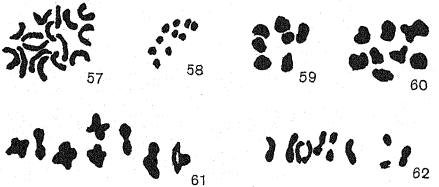
Very few species are in cultivation, and the cytology of the genus is therefore very incompletely known, only about a dozen species belonging to five sections having been examined.

There are at least three basic numbers occurring in the genus—7, 8 and 10. It is noteworthy that no polyploids have been observed.

The somatic chromosomes of *I. Holstii* and *I. Sultani*, as well as those figured by Heitz (1929), are similar to those of *Geranium*.

Meiosis has been studied mainly in *Impatiens Sultani*. The configurations at metaphase are comparable with those of *Limnanthes* but the chromosomes are shorter and more difficult to observe. The

commonest type of bivalent is that with a single interstitial median chiasma (Fig. 61). In *Impatiens Balsamina* and *I. Roylei* terminalization is much more complete, these species being more comparable with *Geranium* (Fig. 62). No confirmation has been obtained of Nakamura's (1936) observation that the bivalents in the former species are normally all ring-shaped at metaphase.



Chromosomes of *Impatiens*

Fig. 57. *I. Sultani* mitotic metaphase.
Fig. 58. *I. Roylei*.
Fig. 59. *I. Balsamina*.
Fig. 60. *I. Sultani* all first division metaphase, polar view.
Fig. 61. *I. Sultani* first division metaphase, spindle view.
Fig. 62. *I. Roylei* first division metaphase spindle view (1 bivalent is missing).

When the cytology of the genus is better known it should prove of value in the classification of the genus, which is at present unsatisfactory. *Impatiens Sultani* and *I. Holstii*, both of which have $n=8$, are probably allied. The reported occurrence of $n=10$ in four species of *Brachycentron* is also noteworthy.

LINACEAE

Linum

Sect. **Eulinum** Planch.	*n*	*2n*	
L. grandiflorum Desf. (Fig. 64)	8	.	de Vilmorin & Simonet
	8	.	Warburg
	(9)	.	Kikuchi
	.	16, 17	Martzentitzina
L. hirsutum L.	8	.	de V. & S.
	8	.	Fischbach
L. viscosum L.	8	.	Fischbach
L. alpinum L.	9	.	de V. & S.
	18	36	Kikuchi
L. altaicum Fisch.	9	18	Kikuchi
L. austriacum L. (Fig. 63)	9	18	Warburg
	9	.	Freiburg
	27/2	.	Freiburg
L. extraaxillare Kit.	9	18	Kikuchi
L. hologynum Reichb.	9	18	Kikuchi
L. Lewisii Pursh.	9	18	Kikuchi

	n	*2n*	
L. muelleri Moris	9	18	Kikuchi
L. narbonense L.	9	18	Kikuchi
L. perenne L.	9	.	de V. & S.
	.	18	Martzentitzina
	9	18	Kikuchi
L. perenne L. var. *asiaticum*	9	.	Dillman
L. punctatum Presl.	.	18	Martzentitzina
L. salsoloides Lam.	9	.	de V. & S.
L. sibiricum DC	9	18	Kikuchi
L. tenuifolium	9	.	de V. & S.
	.	18	Martzentitzina
L. angustifolium Huds.	15	.	de V. & S.
	.	32	Martzentitzina
	15	30	Kikuchi
L. usitatissimum	15	.	de V. & S.
L. usitatissimum	15	30	Kikuchi
L. usitatissimum (1 strain)	.	30	Emme & Schepeljeva
L. usitatissimum (several strains)	.	32	Emme & Schepeljeva
	.	32	Martzentitzina
L. monogynum	43?	86?	Kikuchi
Sect. **Linastrum**			
L. strictum L.	9	.	de V. & S.
L. maritimum L.	10	.	de V. & S.
L. corymbiferum Desf.	15	30	Kikuchi
	.	18	Martzentitzina
Sect. **Cathartolinum**			
L. Catharticum L.	8	.	de V. & S.
	.	57	Martzentitzina
L. rigidum Pursh.	15	.	Dillman
L. sulcatum Riddell	15	.	Dillman
Sect. **Syllinum**			
L. capitatum Kit.	12	24?	Kikuchi
L. campanulatum L.	14	.	de V. & S.
L. flavum L. (Figs. 65, 66)	15	.	Warburg
	15	30	Kikuchi
	.	30?	Martzentitzina

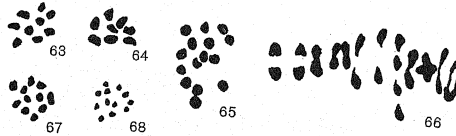
Of this family of twenty-two genera, the only one of which anything is known cytologically is *Linum*. According to Winkler (1931), about 200 species of the genus are known, mainly from the Mediterranean region and western North America. Chromosome counts are recorded for between twenty and thirty species by various authors, mainly by Kikuchi, to whose papers the writer has not had access, de Vilmorin and Simonet (1927), whose paper is merely a list of numbers, and Martzentitzina (1927) who studied the somatic chromosomes of ten species.

Considerable doubt exists about the number in a good many of these, owing to disagreement between different authors. A great confusion of nomenclature also exists in gardens, most of the plants supplied under various names being *L. usitatissimum*, *L. flavum* and *L. austriacum*.

The cytological relationships within the genus are therefore not very clear, but the recorded numbers for the section *Eulinum* correspond with the morphological divisions as follows:

(1) *L. grandiflorum*. An annual species with red flowers, $n=8$.

(2) *L. hirsutum* and *L. viscosum*. Perennial hairy species with blue or purple flowers and ovate leaves, $n=8$.



Chromosomes of *Linaceae* and *Lygophyllaceae*

Fig. 63. *Linum austriacum* first division anaphase.
Fig. 64. *L. grandiflorum* second division metaphase.
Fig. 65. *L. flavum* first division metaphase, polar view.
Fig. 66. *L. flavum* first division metaphase spindle view.
Fig. 67. *Lygophyllum Fabago* second division metaphase.
Fig. 68. *Peganum Harmala* second division metaphase.

(3) *L. austriacum* and several other species. Perennial species with linear leaves and blue, pink or white flowers, glabrous or nearly so, $n=9$ (one triploid and one tetraploid also recorded).

(4) *L. angustifolium* and *L. usitatissimum*. Annual or biennial species with smaller pale blue flowers. $n=15$ or 16. Somatic chromosomes smaller. Emme and Schepeljeva (1927) record both 15 and 16 in different strains of *L. usitatissimum* and both numbers are also recorded by different authors of *L. angustifolium*. Whether both numbers really occur in the latter species it is at present impossible to say.

(5) *L. monogynum*. A perennial white-flowered species from New Zealand, $n=43$?

For the other sections the facts are too doubtful for discussion (e.g. for *L. catharticum* $n=8$ and $2n=57$ are recorded by different authors). The reported occurrence of $n=15$ in all three sections is, however, noteworthy. These sections, in general, consist of yellow-flowered species, in contradistinction to *Eulinum*, and it is possible that $n=15$ is basic for this part of the genus.

The present writer has examined the somatic chromosomes of *L. austriacum* and the meiotic chromosomes of *L. grandiflorum*, *L. austriacum* and *L. flavum*. The chromosomes are very closely

comparable with those of the Geraniaceae. In general they are completely terminalized at diakinesis and at metaphase, and show either one or two terminal chiasmata. In *L. flavum* occasional interstitial chiasmata have been observed at metaphase (Fig. 66).

The peculiar relations which seem to occur in this genus in respect of chromosome number and the absence of cytological information for other genera of the family, render speculations as to phylogeny within the family or in its relation to other families, impossible at present.

ZYGOPHYLLACEAE

Of the twenty-six genera of the family, the writer has examined one species of each of two, no previous counts having been recorded. These are *Zygophyllum Fabago* and *Peganum Harmala*, which are widely separated genera in Engler's (1931) classification. The former has $n=11$, the latter, which has the smallest meiotic chromosomes of any of the plants examined, $n=12$. No satisfactory observations of chiasmata have been obtained for either species. *Peganum* has by some authors been included in the Rutaceae, but all the counts given by Tischler (1927) for this family have $f=9$. It seems therefore best excluded from it. Nothing further can be said of the relationships of the group.

It may be noted that in the Zygophyllaceae and Linaceae the reduction division in the anthers takes place at a much later stage than in the other families considered in this paper.

THE INTERRELATIONSHIPS OF THE FAMILIES CONSIDERED IN RELATION TO CYTOLOGY

It will be first desirable to consider the variations found in the cytological characters, given on pp. 4–6[1], within the group.

(1) *Chromosome number.* This shows considerable variation in all the genera studied.[2] It has, however, been found possible in most of them to suggest a fundamental basic number. These are as follows:

Geranium	7 or 14
Erodium	10
Pelargonium	9
Oxalis	7
Tropaeolum	12 or 14
Limnanthes	5

[1] Part I of this paper.

[2] Except the small genus *Limnanthes* (and those of which one species only has been counted).

For the genera *Impatiens* and *Linum* it is at present uncertain what is the original number or whether this can be determined. The evidence points to eight for *Linum* and perhaps seven for *Impatiens*.

(2) *Chromosome morphology.* No striking variations have been observed either within or between the species examined. No trabants have been noted.

(3) *Size of chromosome complement.* Variations occur in *Geranium* and in *Linum* and in the meiotic chromosomes of *Impatiens*. The chromosomes of *Limnanthes* are strikingly large, being approached only in two or three species of *Geranium*, of which only the somatic chromosomes have been observed.

(4) *Behaviour of chiasmata.* All the plants studied fall into Darlington's (1937) group 3 with incomplete terminalization, but may be divided as follows:

(*a*) Terminalization nearly complete:

Geranium
Erodium
Pelargonium
Oxalis
Tropaeolum
Linum
Impatiens Roylei

(*b*) Terminalization not more than 60 or 70 % at metaphase:

Impatiens Sultani
Limnanthes

(5) Behaviour at meiosis. Secondary association is doubtful. Irregularities in meiosis have been observed in *Geranium "platypetalum"* only. Nakamura (1936) has observed irregularities in *Impatiens Balsamina* under the influence of external conditions.

The most useful characters in the order are therefore chromosome number and chiasma formation and, to a lesser extent, chromosome size.

Of the families considered Geraniaceae, Oxalidaceae and Tropaeolaceae show a close resemblance in all these respects, for though the three genera of the Geraniaceae have different fundamental numbers, *Geranium* is to be considered the most primitive for the reasons given earlier. The close relationship between these families is therefore confirmed on cytological grounds. The essentially South American distribution of these families as compared with the others considered, is additional evidence of their closer relationship.

Limnanthes differs from these families markedly in all three respects. The basic number 5, which is lower than any other found in the order, the large chromosomes, and the interstitial chiasma make it very distinct. The relationship between the Limnanthaceae and the three preceding families cannot therefore be considered to be close.

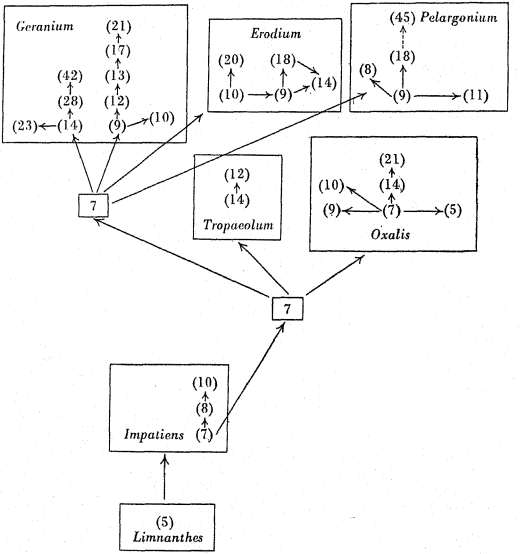


Diagram 2. This diagram is to illustrate the possible relationships of the families and genera in the Geraniales as suggested in the last section if hypothesis (2) (p. 208) is followed. The diagram has been simplified and it is not suggested that *Impatiens* is actually derived from *Limnanthes* nor the other genera from *Impatiens*.

Impatiens is in some respects intermediate. The basic number is doubtful, but $n=7$ certainly occurs. The chromosomes are variable in size, but the somatic chromosomes in the species known are not different from the Geraniaceae except in their better marked con-

striction. The chiasma formation is intermediate, both types being found in different species.

Two possible hypotheses may be suggested:

(1) That Limnanthaceae and Balsaminaceae are not related to the other three families, and are rightly placed by Engler in the Sapindales.

(2) That an evolutionary trend exists leading from the Limnanthaceae through the Balsaminaceae to the other three families, this being accompanied by increase in chiasma terminalization and increase in chromosome number from five to seven (and to more within the families). This is shown in tabular form in Diagram 2. The reverse process is considered to be unlikely. It is noteworthy that the Limnanthaceae are considered primitive by Hutchinson.

Linaceae and Zygophyllaceae have been excluded from the above discussion as too little is known of them. The absence of $n=7$ suggests a relationship at any rate less close than that between the Oxalidaceae, Tropaeolaceae and Geraniaceae.

SUMMARY

1. The use of cytological characters as taxonomic criteria is discussed.

2. Examinations of the somatic and meiotic chromosomes of numerous members of the Geraniales have been made.

3. In the genus *Geranium* the perennial species show polyploidy, the annual species aneuploidy. Aneuploidy also occurs in two perennial species. Certain size variations occur.

4. The classification of *Geranium* is discussed.

5. A comparison is made between *Geranium* and other genera of flowering plants.

6. In *Erodium* diploids and tetraploids occur in most of the subsections.

7. The subsection *Absinthoidea*, the species of which are dioecious, differs in basic number from the other species of *Erodium*.

8. *Pelargonium* has a different basic number from *Geranium* and *Erodium*, and higher polyploids occur.

9. The relationship of Geraniaceae, Oxalidaceae and Tropaeolaceae is confirmed as probable on cytological grounds.

10. Balsaminaceae and Limnanthaceae show certain cytological differences as compared with the above families, and are less closely related or quite unrelated.

11. The position of Linaceae and Zygophyllaceae is doubtful.

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THE INTAKE OF IONS BY CARROT TISSUE AT DIFFERENT HYDROGEN-ION CONCENTRATIONS

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(From the Cambridge University Botany School)

(With 14 figures in the text)

Introduction

In previous publications it has been pointed out that the Donnan equilibrium must be expected to play a part in determining the distribution of ions between living cells and their environment: in the phases of the cytoplasm are indiffusible ions, perhaps of both charges, and between these and the external medium diffusible ions will tend to become distributed in the manner characteristic of that state (Briggs & Petrie, 1928, 1931). There are in addition other determinants of the distribution of ions between cells and their surroundings: apart from chemical fixation and adsorption, there is a mechanism that causes ions to be accumulated in the vacuole (Hoagland & Davis, 1929; Brooks, 1929; Briggs, 1930). It has been suggested (Briggs, 1932) that in mature cells anions so accumulated in the vacuole are exchangeable for other anions in the external solution, while in the cytoplasm both anions and cations can be exchanged; but, while exchange in the cytoplasm appears to be rapid, probably that in the vacuole is exceedingly slow.

It is possible that the Donnan system may reveal itself in characteristic manner in observations on the intake of ions by such mature cells from solutions of different hydrogen-ion concentrations. The Donnan equilibrium will govern not only the exchange of ions in the cytoplasm, but also the exchange of anions in the vacuole; but the indiffusible cations in the vacuole are mainly those of completely ionized salts, and therefore will be unaffected in concentration by changes in the concentration of hydrogen-ions, unless such changes affect the permeability of the vacuolar membrane. If changes in hydrogen-ion concentration of the external solution caused the

tissue to commence on a new phase of growth, there would be further accumulation of ions in vacuoles; but otherwise it follows that such changes may affect only the distribution of ions between the external medium and the cytoplasm.

Some preliminary observations on the effect of such changes are considered in the present paper in relation to the Donnan system, and other aspects of the problem of ion intake. Most of the experiments were carried out in 1927–9, during the writer's tenure of an 1851 Exhibition Scholarship. Two further experiments were carried out in this School in 1937, while the writer was on the staff of the Waite Institute, University of Adelaide. The investigation is still incomplete, but on account of lack of opportunity to carry it further, it is recorded at its present stage.

The writer is greatly indebted to Mr G. E. Briggs for valuable suggestions during the performance of the experimental work and for critical advice on the preparation of the manuscript.

EXPERIMENTAL

The technique adopted has been used by many workers in experiments with carrot tissue. Disks, 1 mm. in thickness and 17·5 mm. in diameter, were cut from the central region of carrot roots. They were washed in running tap water for about 12 hr. and then for several hours in successive changes of distilled water.[1] After washing, the disks were drained on filter paper, and then placed in the experimental solutions in the ratio of 40 disks to 100 ml. of solution.

Experiment 1. *Ammonium and sodium chlorides*

In this experiment intake from 0·05 *M* solutions of these two salts was observed simultaneously on comparable samples of carrot tissue. A volume of 500 ml. of each of the solutions was prepared, adjusted to *p*H 7·0 with NaOH-NaH_2PO_4 buffer mixture, of which the total concentration in the solution was 0·033 *M*: in the NaCl solution, while the concentration of Cl' was 0·05 *M*, that of Na' was thus 0·083 *M*. The solutions were placed in large stoppered vessels, each with 200 disks of carrot tissue. The temperature was maintained constant at 3° C. and the vessels were agitated continuously on a

[1] This procedure, which is necessary for washing surface detritus from cut tissues, causes an alteration in the cells due to leaching (Briggs, 1932).

mechanical shaker.[1] Samples of solution were removed at intervals for analysis, a sufficient number of disks being removed at the same time to keep the ratios of the volumes of tissue and solution constant. Sodium was estimated as pyroantimonate, ammonium colorimetrically after nesslerization, and chlorine by Volhardt's method. The results of this experiment are recorded in Fig. 1. The final *p*H value of the ammonium chloride solution was 6·1, of the sodium chloride solution, 6·4.

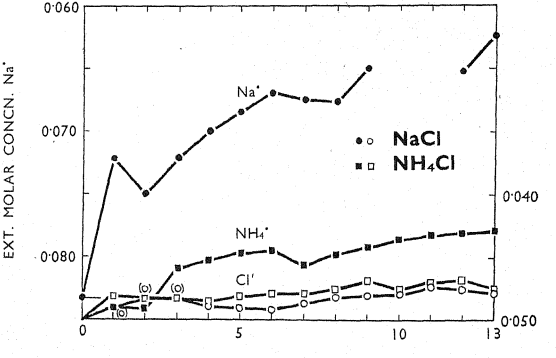


Fig. 1. Curves showing the progress of intake of 0·05 *M* NaCl and 0·05 *M* NH_4Cl from 0·033 *M* Na buffer solution (Exp. 1).

Experiment 2. Ammonium chloride

A similar experiment with 0·05 *M* NH_4Cl was carried out at three different *p*H values in presence of the same buffer as that used in Exp. 1; the concentration of $Na^{\cdot}$ in each solution was 0·033 *M*, and the temperature again 3° C. In addition to the estimation of $NH_4^{\cdot}$ and Cl′, the *p*H of the solution was measured periodically by the

[1] Steward (1931) has criticized results obtained on the absorption of solutes under conditions of limited oxygen supply. But in the present experiment the air supply to all samples was almost identical; the results within one experiment are therefore comparable and refer to the behaviour of carrot tissue under a given set of conditions. The conditions inducing maximum rate of intake are arbitrary, and are often not achieved in the living plant.

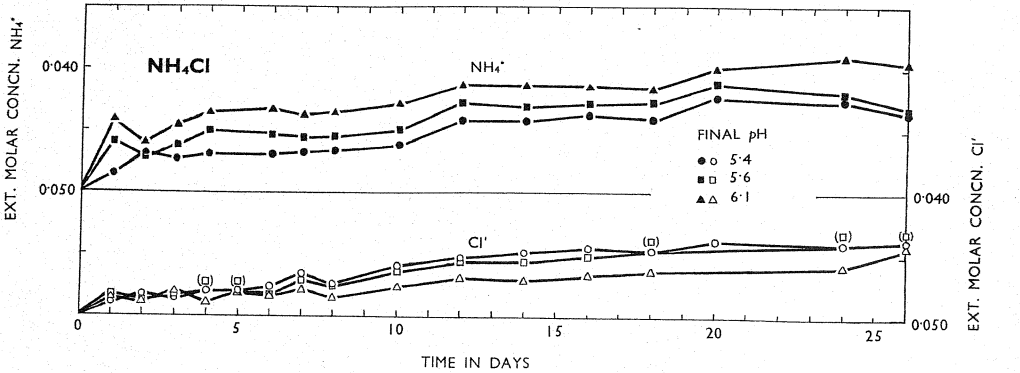


Fig. 2. Curves showing the progress of intake of the ions of 0·05 *M* NH_4Cl from constant sodium buffer solutions (0·033 *M* Na) at three different *p*H values (Exp. 2, Table I).

indicator method. The results are expressed in Table I and Figs. 2 and 3.[1]

After the conclusion of this experiment on the 26th day the solutions were somewhat turbid. The tissue was removed from the sample of medium *p*H by straining through cotton-wool, and the $NH_4^{\cdot}$ content determined. Shaking was then continued for 4 days, after which the $NH_4^{\cdot}$ content was found to be unchanged. This

TABLE I. *p*H *drift in Exp.* 2

Time in days	Solution 1	2	3
0	5·0	6·3	7·0
1	5·0	6·1	6·8
2	5·0	5·9	6·6
3	5·0	5·9	6·6
4	5·1	5·9	6·5
6	5·2	5·8	6·5
10	5·4	5·7	6·3
26	5·4	5·6	6·1

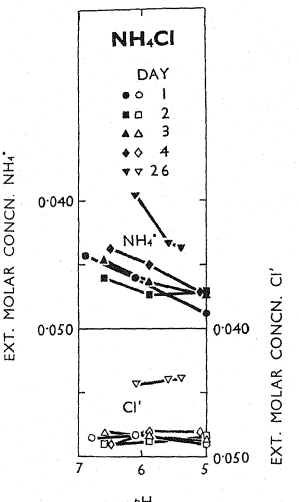


Fig. 3. Curves showing the relation of the external concentrations of the ions to the external *p*H in the absorption of 0·05 *M* NH_4Cl at various points in time; from the data illustrated in Fig. 2 (Exp. 2, Table I).

suggests that the removal of ammonium from the solution by any means other than absorption by the tissue was unlikely even in the turbid solution. A loss of NH_3 to the gas phase was of course possible, and this would be greater the more alkaline the external solution. That this loss was probably small is shown by the very slight changes even in the most alkaline solutions between 12 and 18 days.

[1] In Fig. 3 and in a number of others, external concentration has been plotted against external *p*H. The ordinates in such case are not a measure of intake as a function of *p*H alone, since the greater the intake the smaller the external concentration; for constant external concentration the curves of intake against *p*H would be steeper.

Experiments 3–6. *Ammonium chloride*

For purposes of confirmation and comparison a number of experiments were carried out in which the intake was determined only on the fourth day. In these cases the samples consisted of 100 ml. of the solution, buffered as before, and containing 40 disks of carrot tissue. They were maintained at 4° C., shaken continuously, and the stoppers removed several times during the daytime. The results of these experiments are given graphically in Figs. 4 and 5.

Experiments 7–9. *Ammonium sulphate*

Further experiments on the intake after 4 days were carried out with ammonium sulphate, in experiments similar to 3–6. In Fig. 6 is given a set of results with 0·05 *M* $(NH_4)_2SO_4$ and in Fig. 7 are given two sets with 0·025 *M* $(NH_4)_2SO_4$. Sulphate was estimated as the barium salt. The temperature in each case was 4° C.

Experiment 10. *Sodium chloride*

One experiment with sodium chloride has already been described above. In Table II and Figs. 8 and 9 are given the results of another experiment carried out at four different *p*H values with 0·05 *M* sodium chloride in presence of 0·033 *M* KOH-KH_2PO_4 buffer; this

TABLE II

*p*H *drift in Exp.* 10

	Solution			
Time in days	1	2	3	4
0	4·35	6·02	6·97	8·05
1	4·69	5·91	6·77	7·32
2	4·83	5·89	6·71	7·21
4	4·97	5·77	6·53	7·03
6	5·20	5·77	6·51	6·94
8	5·19	5·75	6·37	6·80
21	5·32	5·68	6·22	6·64

had a K-ion concentration that was the same at the outset for all solutions. The temperature was 3° C. In this experiment the apparatus was not shaken as in those with ammonium chloride, but a stream of air, laden with water vapour, was bubbled through the liquid in the containing vessels. By this means it was expected that the gradients due to gaseous exchange would be broken up and the

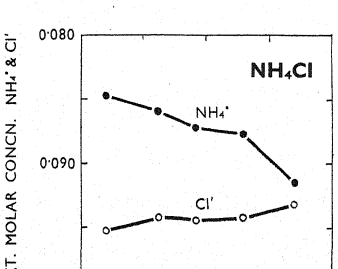


Fig. 4. Curves showing the relation after 4 days of the external concentrations of the ions to the external *p*H in absorption of 0·1 *M* NH_4Cl from constant Na buffer solutions (Exp. 3).

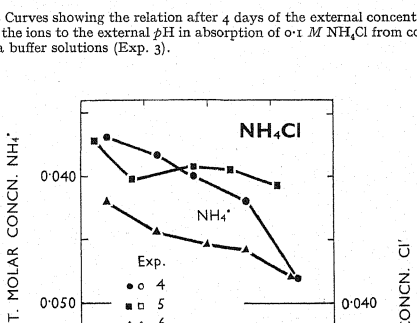


Fig. 5. Curves showing the relation after 4 days of the external concentrations of the ions to the external *p*H in the absorption of 0·05 *M* NH_4Cl from constant Na buffer solutions (Exps. 4–6).

carbon dioxide carried away more efficiently than in previous experiments. A small amount of evaporation may have occurred on account of the air stream not being initially saturated. Unlike the

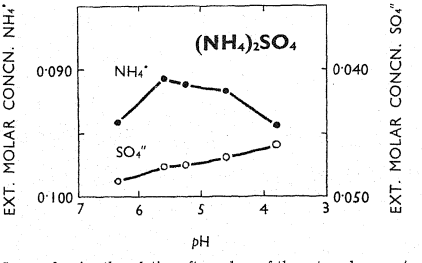


Fig. 6. Curves showing the relation after 4 days of the external concentrations of the ions to the external pH in the absorption of 0·05 M $(NH_4)_2SO_4$ from constant Na buffer solutions (Exp. 7).

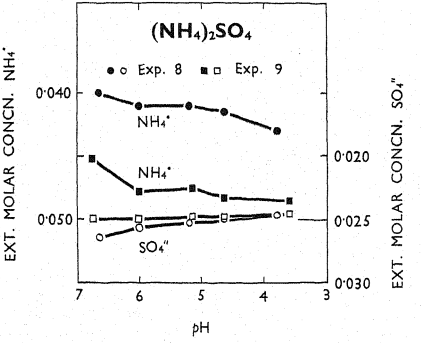


Fig. 7. Curves showing the relation after 4 days of the external concentrations of the ions to the external pH in the absorption of 0·025 M $(NH_4)_2SO_4$ from constant Na buffer solutions (Exps. 8–9).

experiments with ammonium salts, this experiment was accompanied by no risk of loss of the estimated substances to the atmosphere. The pH was estimated by means of the quinhydrone electrode.

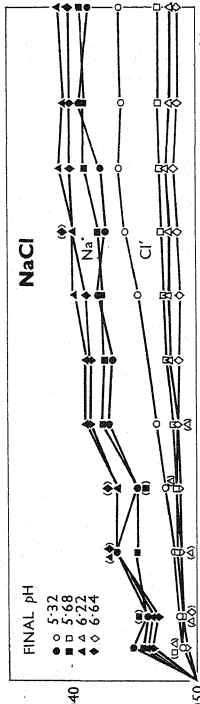


Fig. 8. Progress curves for the intake of the ions of 0·05 *M* NaCl from constant potassium buffer solutions (0·033 *M* K) at four different *p*H values (Exp. 10).

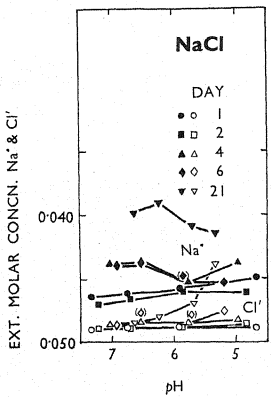


Fig. 9. Curves showing the relation of the external concentrations of the ions to the external *p*H in the absorption of 0·05 *M* NaCl at various points in time; from the data illustrated in Fig. 8 (Exp. 10).

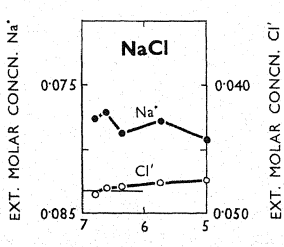


Fig. 10. Curves showing the relation after 4 days of the external *p*H in the absorption of 0·05 *M* NaCl from constant sodium buffer solutions (total Na = 0·0833 *M* at commencement) (Exp. 11).

Experiment 11–13. *Sodium chloride*

Some further experiments were carried out in which the intake of sodium chloride was determined after 4 days. In Fig. 10 (Exp. 11) are shown results with 0·05 *M* NaCl in NaOH-NaH_2PO_4 buffer whose

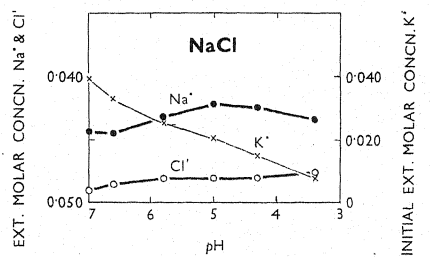


Fig. 11. Curves showing the relation after 4 days of the external concentrations of the ions to the external *p*H in the absorption of 0·05 *M* NaCl from constant phosphate (varying potassium) buffer solutions (Exp. 12).

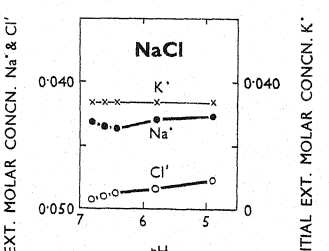
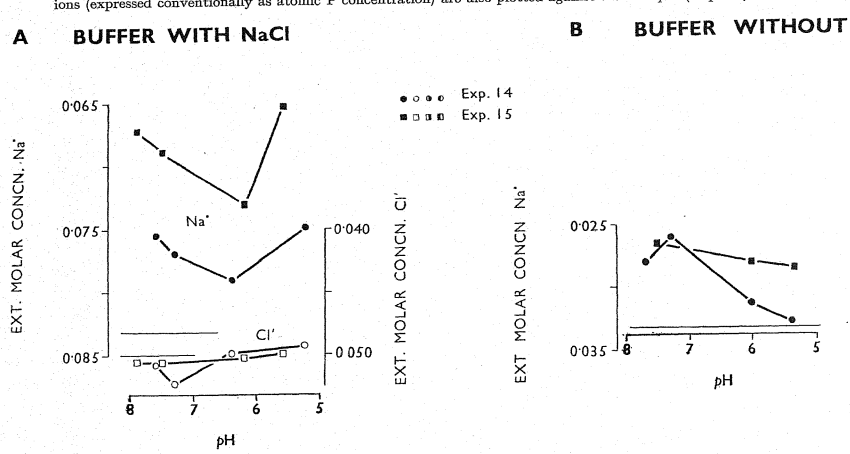


Fig. 12. Curves showing the relation after 4 days of the external concentrations of the ions to the external *p*H in the absorption of 0·05 *M* NaCl from constant potassium buffer solutions (0·033 *M* K) (Exp. 13).

Na-ion concentration was 0·033 *M* at all *p*H values. In Fig. 12 (Exp. 13) are shown the results of a similar experiment with KOH-KH_2PO_4 buffer in which the initial K-ion concentration was constant at 0·033 *M*. In these experiments the vessels were shaken continuously. The temperature was 3° C.

Fig. 13. Curves showing *A*, the relation after 24 hr. of the external concentrations of ions to the external *p*H in the absorption of 0·05 *M* NaCl from constant buffer solutions (total Na = 0·0833 *M* at commencement); and *B*, the same relationship for certain of the ions of the buffer solutions in the absence of the NaCl; in both cases initial concentrations of the total phosphate ions (expressed conventionally as atomic P concentration) are also plotted against the final *p*H (Exps. 14 and 15).



EXT. CONCN. P
EXP. 14
0·030
0·020
0·010
INITIAL
FINAL
5
6
7
8
pH
EXT. CONCN. P
EXP. 15
EXT. MOLAR CONCN. HPO4"
EXP. 14
EXT. MOLAR CONCN. HPO4"
EXP. 15

EXT. CONCN. P
EXP. 14
0·030
0·020
0·010
INITIAL
FINAL
5
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pH
EXT. CONCN. P EXP. 15
EXT. MOLAR CONCN. HPO4" EXP. 14
EXT. MOLAR CONCN. HPO4" EXP. 15

Experiment 14–15. *Sodium phosphate buffer with and without sodium chloride*

In these experiments the interactions of various ions initially present were observed by measuring intake after 24 hr. at 10° C. from 0·033 *M* $NaOH\text{-}NaH_2PO_4$ buffer solution with and without the inclusion of 0·05 *M* NaCl. The disks were washed for 4 days in running tap water before commencing the experiment. Solutions of four different *p*H values were used (except in one series where there were only three). Sodium was estimated as sodium-uranyl-magnesium acetate (Piper, 1932), phosphate by the Briggs method and chlorine by the electrometric method of Best (1929). The *p*H was measured by the quinhydrone electrode. The solutions were aerated in series by a constant stream of air which was freed from carbon dioxide between each vessel. The two experiments were identical except for a possible difference in the aeration. The results are given in Fig. 13.

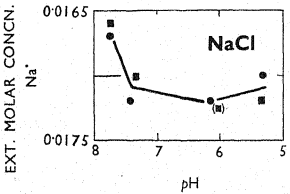

Fig. 14. Curves showing the relation after 4 min. of the external concentrations of ions to the external *p*H in the absorption of 0·010 *M* NaCl from constant medium buffer solutions (total Na = 0·017 *M* at commencement) (Exp. 16).

Experiment 16. *Sodium chloride*

In this experiment the solutions consisted of 0·010 *M* NaCl in 0·007 *M* $NaOH\text{-}NaH_2PO_4$ buffer at four different *p*H values. Eighty disks, after being washed for 4 days in running tap water, were dropped into 200 ml. of each solution, which was gently agitated and then decanted after 4 min. at 19° C. The intake of sodium was then estimated. The results are given in Fig. 14. The experiment was performed in duplicate.

Discussion of results

The progress curves

The curves for ammonium chloride (Figs. 1 and 2) show a rapid initial intake, usually followed, in the case of NH_4-ions, by a loss from the tissue, or by a cessation of intake, on the second day. In two instances there is a loss of Cl-ions also, but the Cl-ion curves are irregular during the first few days, and it is questionable whether the irregularities are significant as regards ion intake. After the second day intake of both ions proceeds at a much slower and fluctuating rate. There is no clearly defined point at which equilibrium is reached, although in Fig. 2 there is shown an increase of the amount of NH_4-ions in the external solution towards the 26th day.

The sodium chloride curves (Figs. 1 and 8) resemble those of ammonium chloride; there is a loss of the cation on the second day, which is followed by a further slow, prolonged and irregular intake.

The loss of cations from the tissue during the early part of the experiments will be discussed in the next section; here we shall consider the remaining general features of the progress curves. When tissue is transferred to a solution containing ions, these probably first enter and approach equilibrium in certain phases of the superficial cells in which rapid exchange is possible. Approach to equilibrium in the internal cells depends on passage of ions through or around the superficial cells, and is probably slower. Two additional processes may be superimposed on these. Firstly there is the further accumulation of ions by the vacuoles of all cells. Secondly there is the accumulation of ions by new cells forming at the surface of the disks, or by new material forming within existing cells. Formation of such cells or material, from reserves brought from the interior of the tissue, is probably associated with an increase in the total protein content of the disks (Steward *et al.* 1932). Probably equilibration in the internal cells is the process that is chiefly responsible for the intake of ions in the first few days of the experiment; the subsequent slower intake may be mainly due to accumulation and growth. Continuous chemical transformation also may contribute to the intake of NH_4-ions, but it is less likely to play an appreciable part in the cases of the other ions.[1]

[1] It is remarkable that in Exp. 1, where the conditions for the two salts were comparable, Na^+ was taken in at a relatively greater rate than NH_4^+.

It is possible that some of the irregularities in the progress curves may be due to variation in the rate of oxygen supply, which, as Steward (1931) has found, is a factor determining the rate of intake of ions by the tissue. In the present instance such an effect may have been due to the relation of oxygen supply to growth rate.

The final increase in the NH_4-ion concentration of the external solutions, shown in Fig. 2, may be due to breakdown of cells. We can calculate "average" internal concentrations of NH_4-ions at this stage from the volume of the tissue (which is known approximately) and from the amounts of NH_4-ions taken in; we then find that, unless a considerable amount of NH_4-ions is fixed in the tissue, the internal concentrations must have considerably exceeded the external. If breakdown of cells occurred, the internal concentration would tend to come to the same value as the external, so that NH_4-ions would tend to pass out of the tissue, except in as far as they were bound by non-mobile anions.

Reference should perhaps be made at this point to the drift of respiration rate of the tissue. This rate was probably at a maximum early in these experiments as in those of Briggs & Petrie (1931), and may have fallen progressively for a number of days thereafter. Briggs & Petrie have shown that respiration rate may be a factor controlling the distribution of ions between the tissue and the external medium; but the absolute amount of ions that would move in or out of the tissue, as a result of the effect of changes in the respiration rate on the dissociation of cytoplasmic colloids, is probably very small in comparison to the amounts of ions taken in by the tissue in the present experiments.

The relation of cation intake to pH and its drift with time

Had the Donnan equilibrium manifested itself in the relation between the amount of ions taken in and the *p*H of the external solution, we should expect that, when equilibrium was attained, more cations and fewer anions would be taken in, the greater the *p*H value. While the relationship to *p*H of the amount of ions taken in is sometimes of this type in the data, at other times it is clear that other factors must play a part in determining it. In fact the drift with time of the relationship is complex; we shall attempt, however, to define some general features characterizing it.

Let us consider the results for the ammonium salts first. Fig. 3

depicts the relation of intake to *p*H at a series of points in time. Examination of this figure enables us to differentiate three successive stages through which the relationship passes. The transitional periods may have characters that are not revealed by these data, or in other words there may be other characteristic stages not here apparent. On the first day we see what we shall call the first stage, with greater intake of NH_4-ions the greater the *p*H. In the second stage on the second day there has been further intake from the most acid solution but loss into the others, resulting in a **V**-shaped curve, with minimum lying between *p*H's 5·1 and 6·6. The third stage on the third day results from a possibly insignificant loss from the tissue in the most acid solution, and an uptake again in the others, so that the relationship to *p*H is again that of the first stage. This relationship then persists till the end of the experiment with the continuous uptake referred to in the previous section.

The Cl-ion progress curves, as already pointed out, fluctuate in a possibly insignificant manner during the early portion of the experiment, but soon a relation becomes manifest in which uptake is less the greater the *p*H of the solution, and this relation persists.

The times at which the stages of cation intake are attained will almost certainly depend on the conditions of the experiment; in examining the results of the other experiments we may therefore expect to find some of these stages illustrated, but we can place no significance on their time of manifestation. In Figs. 4, 5 (Exp. 4 and 6) and 7, where the NH_4-ion intake is greater the greater the *p*H, stage 3 is probably exhibited. On the other hand in Fig. 5 (Exp. 5) we are probably seeing stage 2; if this is so Fig. 5 (Exp. 5) reveals the fact that there is a descending limb to the right of the curve, giving it a **Λ** form. The minimum here lies between *p*H's 5·2 and 6·8. Fig. 5 (Exp. 6) may indicate a transitional stage between those of Exp. 5 and 4. Fig. 6 illustrates stage 2 with its descending limb on the right, and the minimum here is located at $pH > 5{\cdot}6$. Fig. 7 (Exp. 9) appears to be almost passing out of stage 2, with minimum between *p*H's 5·1 and 6·8.

In all these experiments the anions, whether Cl' or SO_4'', show a smaller intake the greater the *p*H. In the case of Exp. 8 this relation is established by loss of SO_4-ions from the tissue in the more alkaline solutions. This phenomenon could be expected in a Donnan system if indiffusible cations were present in the cytoplasm, and the system had previously approached equilibrium with a high external concentration of SO_4- or H-ions. Under these circumstances the internal

concentration of SO_4-ions may have been initially high, and may have had to fall to approach equilibrium with the experimental environment. A more probable explanation would be that, either at all *p*H's or only at the higher ones, breakdown of some cells took place resulting in loss of ions to the external solution.

In the case of the sodium salts the earliest time for which there is information is four minutes from the time of immersion of the tissue in the experimental solution. The 4 min. results given in Fig. 4 show no intake of Na-ions except from the most alkaline solution. There is thus a tendency to exhibit stage 1. Na-ions are lost from the tissue in the acid solutions; this would be expected in a tissue containing indiffusible anions which had previously approached equilibrium with a solution of greater Na-ion or smaller H-ion concentration.

Turning now to Fig. 9, on day 1 we see what may correspond to a stage between 1 and 2: considerable intake has occurred in the most acid solution, but intake is less the greater the *p*H. Loss from all solutions occurs between days 1 and 2; in the ammonium chloride experiment at this time the loss characterized only the more alkaline solutions. On the fourth day intake has taken place from all solutions, but in such manner that stage 2 is now manifested with its **V**-shaped curve, the minimum lying between *p*H's 4·9 and 6·5. It is difficult to judge from the data the extent to which this stage has been attained in different manners in the sodium chloride and ammonium chloride experiments. Stage 3 is revealed on the sixth day. Subsequently intake falls behind in the most alkaline solution, possibly because of some secondary effect resulting from prolonged immersion in the alkaline solution.

Stage 2 is shown in Fig. 10 with minimum between *p*H's 5·7 and 6·6, in Fig. 12 with minimum between *p*H's 5·8 and 6·6, and in Fig. 13 with minimum between *p*H's 5·9 and 7·3. Stage 3 is shown in Fig. 14, again with reduced intake in the most alkaline region. In the results depicted in Fig. 11 another factor is superimposed on those governing intake in the other experiments: increasing numbers of K-ions in the alkaline solution have evidently competed with Na-ions for entry, resulting in a general decrease in amount of Na-ions taken in from these solutions. It would have been interesting had K-ion intake been estimated in all these experiments.

In the sodium chloride experiments the Cl-ion intake shows the same relationship to *p*H as the anion in the experiments with ammonium salts, although in Fig. 13 the relationship is achieved by loss of ions from the tissue.

Viewing the results as a whole, the data for the cations suggest that at certain stages in the process of intake the relationship to *p*H is what would be expected in a Donnan system. When this relationship is manifested, for a given difference in *p*H value the difference in the amount of anions taken up is small in comparison with the difference in the amount of cations taken up; this suggests that the indiffusible ions of the cytoplasm are mainly negatively charged. The divergences from the expected relationship may be partly due to effect of the *p*H of the solution on the permeability of the tissue to ions penetrating into the inner cells; we may thus be observing effects of *p*H on the rate of entry of ions rather than on the equilibrium amounts taken in. The loss of ions from the tissue is difficult to account for. It appears to occur only when the tissue is in buffered solutions: it is not shown in the experiments of Stiles (1924), nor in those of Asprey (1933), where the progress of intake was observed at much shorter intervals of time. It is evident that the drift of the rate of intake of cations is too complex to interpret without further experimental investigation.

The Cl-ion intake throughout shows the relation to *p*H that would be expected with a Donnan system; but this relation can be interpreted in another way also. In Fig. 13 it is shown that there is a loss of Cl-ions from the tissue that is greater the greater the HPO_4-ion concentration of the external solution, while there is a loss of total phosphate that is less the greater that of Cl-ions. This suggests that the tissue is permeable to HPO_4- and not to H_2PO_4-ions, and that the greater the external concentration of HPO_4-ions the more is their passage out of the tissue inhibited, and simultaneously the greater becomes the loss of Cl-ions. It is thus possible that the smaller intake of Cl-ions from the more alkaline solutions in the other experiments is due to competition with the greater number of HPO_4-ions in such solutions. It could also be suggested, as was done for the SO_4-ions, that there is a loss of ions from the tissue due to breakdown of cells; this might be the same at all *p*H's, and be superimposed on a competitive uptake of Cl- and HPO_4-ions.

Drift in the external hydrogen-ion concentration

It will be seen from Tables I and II that there is a drift in the hydrogen-ion concentration of the solutions during the period of immersion of the tissue. When the total change of *p*H of the solution is plotted against the initial *p*H, it is found that the solution whose *p*H would remain unchanged throughout the experiment would in

general be one between 5·2 and 5·4: solutions initially more alkaline or more acid drift with time towards this intermediate value.

Robbins (1923; see also Robbins & Scott, 1925) has found similarly the *p*H value of the solution that remains unchanged in this value when placed in contact with various tissues, and he claims that this represents the "isoelectric point of the tissue". Youden and Denny (1926, 1927) disagree with Robbins: they hold that the change in the external *p*H is brought about by substances (other than carbon dioxide) that emerge from the tissue, and not by the buffering effect of the proteins within. Youden and Denny's experiments, however, were carried out differently from those described in the present work: it is not stated that the tissue was washed after cutting, and the ratio of volume of tissue to that of solution was comparatively very large. It is thus clear that such substances, if they do come out of the tissue, must be of less importance in the present work. But in any case the present data do not enable us to determine the isoelectric point of the tissue. Carbon dioxide is continually diffusing out and this will have an acidifying effect on all solutions in addition to that due to the combination of the proteins with cations. It should also be noted that the acid solutions are made more alkaline by the tissue, even though there is a greater intake of cation than anion (at least as far as concerns the ions measured). Intake of phosphate-ions would, however, tend to make the solution alkaline, and it can readily be shown that this effect would be greater the greater the amount of HPO_4-ions in the external solution, i.e. the more acid the solution. This effect apparently more than counter-balances the acidifying effect of excess absorption of cations over anions.

It is clear that the *p*H of the external solution that is not changed by the tissue is determined by a number of factors.

SUMMARY

If the tendency to establish Donnan equilibrium between cytoplasmic phases and the external medium plays a part in determining the amounts of ions taken up by plant tissue, then in intake from solutions of different hydrogen-ion concentration, provided other factors do not complicate the picture, more cations and fewer anions will be taken in the greater the *p*H of the solution. The present investigation records some preliminary observations on this relation of intake to *p*H. The observations show that for cations the course

of intake is complex, and the relation of the amount of ion taken in to *p*H of the solution drifts with time, although at certain times, especially at the beginning, and again after the lapse of some days, the relation has the form expected in a Donnan system. With anions, while the intake in general is related to *p*H in the expected manner, it is possible that other factors may have been the cause of the relationship having the form observed. Thus, while there is some suggestion that the Donnan system manifests itself in determining the amounts of ions taken up by the tissue at any time, it is evident that other factors play an important part.

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THE RESPIRATORY METABOLISM OF CARROT TISSUE

I. MATERIAL AND METHODS

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(With 7 figures in the text)

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I. Introduction and summary

In an investigation of the relation between respiration and fermentation in the higher plants, the author has carried out a long series of experiments with slices of carrot roots immersed in water. Two kinds of apparatus were used, one a continuous current apparatus in which the carbon dioxide evolved in respiration was swept out of the medium and absorbed in Pettenkofer tubes, the other a closed system in which oxygen uptake and respiratory quotients were measured manometrically. Hitherto the manometric method has been little used in plant physiology, except in work with unicellular organisms. Moreover, no systematic investigation of respiration and fermentation in plant tissue slices has been reported. This introductory paper outlines the method of preparation of the plant

material, and describes the apparatus used. The application of the manometric method to the study of plant tissue respiration is discussed in detail.

The author is greatly indebted to Dr F. F. Blackman for his encouragement and help in the course of these investigations.

II. Plant material

Carrot tissue was chosen, as it had already proved useful in work on permeability carried out by Briggs & Petrie (1931). Preliminary experiments, in the continuous current apparatus, were of long duration, and a tissue was required which could be immersed in aqueous solutions for some weeks without showing marked oxidase reactions or disorganization. Carrot roots give an oxygenase reaction only in their skin, and if slices are kept in well-aerated water they are not readily attacked by bacteria. Well-washed tissue will also live for many hours in nitrogen, when it produces carbon dioxide and alcohol in a ratio very close to that for yeast fermentation. Secondary growth at the cut surfaces has never been observed in tissue kept under water. Thin slices of the tissue retain their turgor, and do not disorganize, even after several weeks' immersion, though it is probable that some surface cells break away. The cells contain very little or no starch, and after the first period of washing they do not lose carbohydrates into the external medium.

The main disadvantage of carrot tissue in work of this kind lies in the heterogeneity of the tissue. As shown in Appendix I, most of the cells of a slice belong to a secondary parenchyma derived from a cambium. Most of these cells correspond in position either to phloem or xylem, while about 2% of each tissue slice is made up of resting cambium, dead xylem elements, and small sieve tubes. The cambium is not active in the stored root, not does it become active in the tissue slice, even if heteroauxin is added.

Thus the cells of a root are not originally homogeneous, and they probably differ even more in some ways after cutting. The outermost cells of a slice must suffer more from the "wound reaction" than the inner ones, and even in thin slices there must be diffusion gradients across the tissue in respect of oxygen concentration or of anything supplied in the medium. There may also be a difference of polarity between the morphologically upper and lower cells. It must therefore be realized that we are dealing with a complex population of cells,

though it is probable that the complexity is little greater than in the apparently homogeneous material of *Chlorella* or yeast cultures.

III. PREPARATION OF THE MATERIAL

In preliminary work, carrots were bought as required, in bunches of about six, and they were probably of very varied origin. Later large numbers of carrots of one variety were obtained every autumn from a farm at Gamlingay, Bedfordshire. In experiments to be described in succeeding papers the origin of the roots is indicated as follows. P. means that they were of the preliminary batches. G. (193–) means that they came from Gamlingay in the October of the year stated.

The roots in each of these yearly collections were sorted and washed free of soil; the tops were cut off, and all diseased roots destroyed. The remainder were grouped in trays in order of size, and stored at 1° C. in a ventilated metal chamber. Carrots from each year's stock, dug in October, usually lasted until July when thus stored. A number of them were attacked by bacteria or fungi, and these roots were removed when infection was obvious. During storage the roots lost water and in time became slightly flaccid. Slices cut from such old roots regained turgor and brittleness on being soaked in water, though this uptake of water often led to troublesome twisting of the slices.

Carrot roots were removed from store as required, in groups of four to six, peeled thickly, and the base and top cut from each. Large roots were cut longitudinally. From the blocks remaining, slices 1 mm. thick were cut, with a modified sledge microtome. The instrument cut the disks accurately to 1 mm. but when they were placed in water slightly greater thicknesses were sometimes reached. In the Pettenkofer experiments, the slices were then cut into four irregular pieces, whose area varied from 2 to 4 sq. cm. In cutting for the manometric experiments, each large slice was laid on a piece of cork, and disks, 0·8 cm. diameter, were cut out with a sharp cork borer (Fig. 1). It was found best to do this immediately after the large slices had been prepared, as these sometimes twisted after washing. Slices obtained in this way suffered less from bruising than when a narrow cylinder of tissue was first removed from a root and then sliced.

If bacterial infection is to be avoided or postponed, it is essential to wash the slices thoroughly to remove the contents of the damaged

cells. In early experiments the slices were washed for a few hours in running tap water, and were then placed in distilled water (100 g. of carrot to about 4 l. of water). This water was changed each day. In all later experiments the initial washing in tap water was prolonged until the slices were required.

It would be a matter of great difficulty to prepare carrot slices under sterile conditions, and to keep them sterile during a long

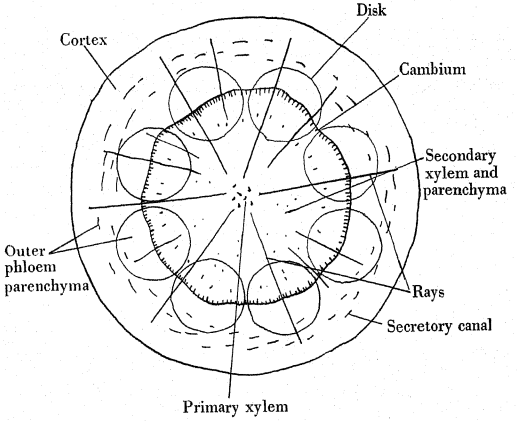

Fig. 1. Diagram of transverse section of carrot root, showing the distribution of the tissues and the method of removal of the disks used in manometric experiments. (× 1·9.)

period of washing, and it is very doubtful whether the trouble involved would be worth while. Cambridge tap water is almost sterile, and slices washed in it are not readily attacked by bacteria. No doubt they bear resting spores, but unless the tissue is injured in some way, as by poisons, or by very prolonged immersion, the bacteria rarely develop so as to contribute to the respiration of the sample. Bacterial contamination did sometimes occur during an experiment. This was always indicated by a clouding of the medium and a rapid increase in the rate of carbon dioxide production. Such experiments were discarded, at least in so far as their later stages were concerned. In

other experiments evidence is available, and will be presented, to show that bacterial respiration or fermentation is negligible. It is particularly important to demonstrate this when the rate of carbon dioxide output is rising, when one might naturally suspect infection.

Most of the experiments to be described have been carried out with the slices immersed in distilled water, or in glucose solutions. In some of the later manometric experiments, buffer solutions were used for special purposes. A few trial experiments and the work of Thomas (1931) made it doubtful whether phosphate buffers have much effect on the rate or drift in the rate of carrot slice respiration. The animal tissues which have been used so much in comparable work, produce acids under certain conditions, and rapidly disorganize in water, and here the use of buffers is essential. The plant tissue of carrot roots, if washed after cutting, retains its nature as a mass of organized cells for many weeks, even in distilled water.

IV. APPARATUS

(1) *The continuous current method: measurement of the output of carbon dioxide*

In many of the experiments to be described, the respiration and fermentation were measured by the absorption, in standard baryta, of the carbon dioxide liberated into the gas stream. The principles of the method are well known. The author used a battery of Pettenkofer tubes with a commutator designed by Dr F. F. Blackman, by means of which a continuous day and night record of the carbon dioxide output could be obtained. Two-, or three-hourly records were taken, and the contents of each Pettenkofer tube were titrated in one operation.

The plant chambers are shown in Fig. 2. The plant slices were placed in 250 c.c. reagent bottles provided with rubber stoppers and inlet and outlet tubes of glass. Each bottle held 200 c.c. of the medium, and between 40 and 50 g. fresh weight of the slices, which moved about slowly in the medium above the bubbler. In a few early experiments 60 or 70 g. of tissue were used, but it is undesirable to use more than 50 g. of carrot in 200 c.c. of liquid. The following was the average ratio in all the experiments to be dealt with.

$$\frac{\text{Volume of medium (c.c.)}}{\text{Fresh weight of slices (g.)}} = 4{\cdot}4.$$

The inlet tube of the vessel passed to the bottom, and was made from a sintered glass bubbler. The gas thus emerged into the medium

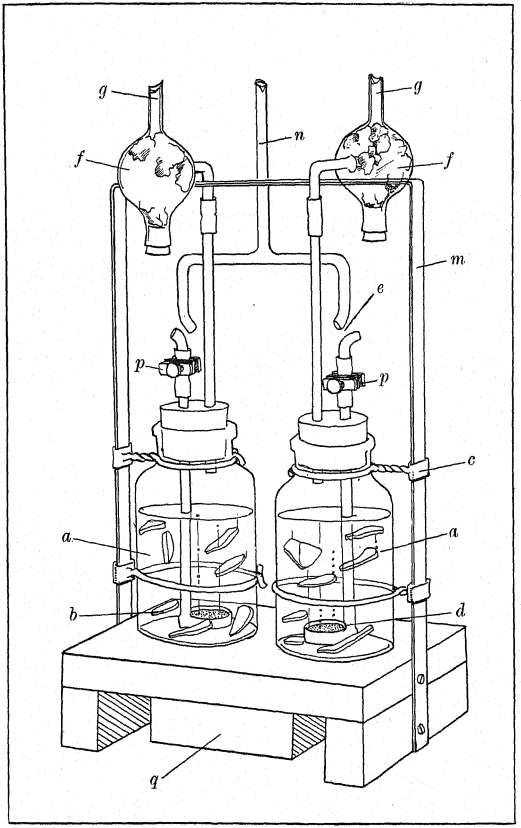

Fig. 2. The vessels of the continuous current apparatus: (*a*) 200 c.c. medium, (*b*) carrot slice, (*c*) wire clips, (*d*) sintered glass bubbler, (*e*) place of insertion of spirals of glass tubing, (*f*) calcium chloride bulbs, (*g*) outlet tubes, (*m*) brass frame, (*n*) inlet tube, (*p*) screw clips, (*q*) lead weight. ($\times \frac{1}{3}$.)

as a stream of fine bubbles which kept the liquid in slow circulation. It was found impossible to suck the gases through the apparatus by aspirators, or a filter pump, without occasionally flooding the tubes with baryta. The gases were therefore driven through the tubes from cylinders. They passed first through long soda lime tubes, and then through glass spirals immersed in the water-bath. The gases used were mainly air, oxygen and nitrogen, which last was obtained almost pure by passing it over hot copper at 400° C. A small bulb of calcium chloride was placed in the circuit between each vessel and the Pettenkofer tubes. The rate of the gas stream was approximately 2 l. an hour, and slight variations in this rate had no measurable effect on the carbon dioxide output.

The respiration vessels were supported in pairs in a weighted wooden frame, sunk in a large screened water-bath, kept at 22·5° C. by means of a mercury-toluene gas thermostat. In most experiments the temperature was observed to vary by not more than 0·2° C. The water-bath was covered by a black cloth, though there was no indication that weak light had any effect on the rate of respiration or fermentation.

The vessels were held in place by thick rings of copper wire, and they were connected to the rest of the apparatus by short flexible rubber joints. It was thus a simple matter to raise the vessels out of the bath for the manipulation of the tissue or medium, and to remove either vessel from the frame. Any fresh supply of medium was added through the bubbling tube, the gas current having been stopped. In fermentation experiments in which iodoacetate was added to the vessels, very little air was introduced with the added medium. After alterations in the medium, the vessels were replaced in the water-bath, and a fresh baryta tube was used for absorption, after about 10 min. had been allowed for the equilibration of temperature and gases.

Twice each day, the vessels were raised from the water and examined for cloudiness or opalescence, as this always accompanied bacterial contamination. It was also necessary sometimes to adjust the rate of the gas current passing through one or other of the vessels, and this was done by means of the screw clips on the rubber connexions at the tops of the inlet tubes.

(2) *The manometric method: the measurement of oxygen uptake and the respiratory quotient*

The Pettenkofer technique was usefully supplemented by a method by which the R.Q. could be measured. On the advice of Dr M. Dixon we chose to use the modified form of the Warburg apparatus, designed by Dickens & Šimer (1930); this has proved highly satisfactory in certain kinds of work with plant tissue.

The method consists in measurement of the pressures set up, by respiratory gas exchange, in a small vessel connected to a constant volume manometer. Readings of pressure changes may be taken every 10 or 15 min., and our experiments rarely lasted for more than 12 hr.: most were concluded within 3 hr. In work on oxygen uptake, or on fermentation, five separate sets of plant material were used at the same time, a sixth vessel acting as thermobarometer. A description of the method may be found in the original paper, and an account of the technique is given in Dixon (1934). The following details are of importance.

(*a*) *Technical details of the apparatus.*

The bath and shaker were of the type described in the paper of Dickens & Šimer (1930). The shaking speed was between 90 and 100 complete oscillations per min., with an excursion of 5 cm. This is recommended by Dickens & Šimer as rapid enough to ensure equilibrium; a more rapid rate of shaking when these vessels are used is, in fact, impossible as there is then great danger of the splashing of baryta into the central compartment. As we used an amount of tissue which gave a much smaller rate of respiration than that used by Dickens & Šimer, it may be taken that the rate of shaking is sufficiently rapid.

A test of the efficiency of the baryta as an absorbent of carbon dioxide was made, by the method of Dixon & Elliott (1930). Absorption of carbon dioxide liberated into the vessel from carbonate was complete in less than 15 min. The baryta is as efficient as the absorbent recommended by Dixon, in which filter paper soaked in strong potash is used.

The temperature of the water-bath was 22·5° C. An efficient thermoregulator was made by replacing the wide bulb of an ordinary mercury-toluene regulator by 18 ft. of thin-walled glass tubing of $\frac{1}{4}$ in. bore, bent so as to pass twice round the bath. The temperature rarely altered by more than 0·2° C. Pressure changes in the control

manometers were due more to changes in barometric pressure than to variation in temperature.

(*b*) *Tissue and medium.*

In most experiments the standard procedure was to use 3 c.c. of medium and 15 of the small carrot slices, 0·8 cm. diameter and 1 mm. thick. The disks were carefully blotted dry and weighed just before each experiment. The fresh weight of 15 disks was very uniform, and was just over 1 g. The medium used was often distilled water. When glucose solutions, or buffer solutions were used, they were freshly made up and sterilized. In any vessel, the maximum rate of carbon dioxide evolution was 250 cu. mm. per hr. Many investigators use much more active samples, and Dixon speaks of 800 cu. mm. per hr. as a high rate.

(*c*) *Measurement of respiration.*

In experiments in which oxygen uptake only was measured, the tissue was placed in the central compartment of the vessel, and 0·5 c.c. of pure *M*/6 baryta in the annular trough. If the experiment was planned to last for more than 3 hr., it was found necessary to supplement the baryta by 0·5 c.c. of 20% potash, placed in the side bulb, with a cylinder of Whatman No. 1 filter paper projecting from it into the vessel. This procedure did not affect the rate of carbon dioxide absorption in the early stages (the baryta being very efficient), but it allowed the efficient absorption of carbon dioxide over a long period.

The vessel constant was calculated from the ordinary formula:

$$K_g = \frac{V_g \cdot \frac{273}{T} - V_f \cdot \alpha_{O_2}}{10{,}000}.$$

In all experiments the value of α_{O_2} for oxygen in water or aqueous media was taken to be 0·03. It was also assumed that the solubility of the gas in the tissue slice was 0·03, and the volume of the slices, approximately 1 c.c. was therefore added to that of the medium and baryta, to give V_f.

(*d*) *Measurement of the respiratory quotient.*

Two methods are available, with the apparatus described, for the determination of the R.Q., and details of them may be found in Dixon's book (1934). The method of Dickens & Šimer (1930) has been used in most of our experiments, and a typical protocol is given in Appendix II.

In this method, two comparable samples of tissue are used, but one is required only for the determination of the "initial" carbon dioxide content of the tissue, medium and baryta. This is accomplished by tipping strong acid into the main compartment of the vessel as soon as this has attained the temperature of the bath. The operation is carried out in a closed system, and the volume of the liberated carbon dioxide is measured by the increase of pressure in the manometer. In a second comparable vessel, the oxygen uptake of the tissue is measured over a period of hours, and then acid is tipped again to liberate the "final" carbon dioxide content. The respiratory quotient is then given by the equation:

$$\text{R.Q.} = \frac{\text{"Final" } CO_2 \text{ content} - \text{"Initial" } CO_2 \text{ content (mm.}^3\text{)}}{\text{Total oxygen uptake (mm.}^3\text{)}}.$$

As Dickens & Šimer point out, the error involved in this determination of the R.Q. is mainly dependent on the amount of the "initial" as compared with the "final" carbon dioxide content. It has been shown for carrot tissue that the carbon dioxide content is low considering the rate of respiration. About half of the "initial" CO_2 measured by the first acid tip is carbon dioxide liberated from the tissue in respiration, and fixed by the baryta, during the initial 10 min. equilibration period. It is not practicable to shorten this period, and in most experiments the measured "initial" CO_2 is of the order of 9–15% of the "final" value—a maximum for which is set by the dimensions of the manometer.

Some direct experiments have been done to determine the real carbon dioxide content of carrot tissue. Using figures obtained by direct acidification of carrot slices in vessels free from alkali, and adding data obtained in R.Q. determinations, we find that the true initial carbon dioxide content of washed tissue slices is of the order of 30–50 mm.^{3} per g. fresh weight. This is the amount of carbon dioxide in tissue in water equilibrated with air. It is equal to the amount which would be dissolved in 1 g. of water under an atmosphere of 3·7–6% carbon dioxide. It is also very approximately equal to the amount of CO_2 evolved from 1 g. of carrot tissue in from 10 to 15 min. at 22·5° C.

There is little difference between the CO_2 content of freshly cut carrot tissue which has been in contact only with distilled water, and that of tissue which has been washed for many days in tap water. The data suggest however that the CO_2 content is proportional to the rate of respiration, although the retention of CO_2 by the tissue as the external CO_2 concentration rises is only slight.

The accuracy of the determination of the "initial" CO_2 in R.Q.

experiments depends largely on the relative rates of respiration by the two comparable tissue samples, and on the rapidity and care with which the baryta is added to the two vessels. Dickens & Šimer state that in their experiments with animal tissues, the "initial" CO_2 content does not exceed 10% of the "final" value. They allow a 20% error in the determination of the "initial" value, and assume an error of 2% in the R.Q. The author has followed them in this respect, though in his work, 20% is probably an overestimate of the error in the determination of "initial" CO_2. In three parallel determinations of this value in paired vessels, the amounts differed by 3·5%, 7·5% and by nil respectively. As, however, the values for "initial" CO_2 in his experiments sometimes exceeded 10% of the "final" values, it seems best to assume that the R.Q. determinations were subject to errors of 2%.

In these experiments, the value of K_g for oxygen is calculated assuming that the solubility of oxygen in water, solutions, and tissue is 0·03 at 22·5° C. Some doubt arises as to the solubility coefficient for carbon dioxide. This gas is liberated into a solution containing HCl, $BaCl_2$, and dead tissue, with or without glucose. The actual strength of the solution was 0·2 *N* HCl, and 0·04 BaCl. A few determinations of α_{CO_2} in this mixture, made by liberating a known amount of carbon dioxide into a closed vessel containing the solution, gave values with a mean of 0·77 (three experiments). This accords well with values given in the International Critical Tables of 0·74 for 0·5 *N* HCl, and 0·73 for 0·2 BaCl. The value actually used was 0·75. Another approximation followed from the assumption that the tissue was, for solubility purposes, 100% water. But this assumption cannot lead to a large error in the R.Q. At a minimum, the water content of the tissue is 90% of the fresh weight. If we correct for this in V_f, the figure calculated for K_{CO_2} is affected by less than 0·5%, and the effect on the R.Q. is negligible.

Table I shows that even if a 10% error arises in the value for α_{CO_2}, the effect on the R.Q. will be within the experimental error. Using average figures for pressure differences, some R.Q.'s have been calculated, assuming arbitrary values for α_{CO_2}. An error of 40% in

TABLE I. *Method of Dickens & Šimer*

α_{CO_2}	Calculated R.Q.
0·90	1·02
0·80	1·00
0·75	0·99
0·60	0·95

the solubility coefficient is thus shown to lead to an error of only 7 % in the calculated R.Q.

The direct method of Warburg (Dixon, 1934) has been used occasionally, partly as a check on the other method, partly because it gives successive values of the R.Q. over short periods of time, which is sometimes required. This method requires strictly comparable samples of material, which are fairly easily obtained by the random sampling of numerous cut disks. In one vessel oxygen uptake is measured, baryta being present. In the other vessel baryta is absent, and the total pressure change may be used to calculate the R.Q., if it is assumed that the oxygen uptake is identical, weight for weight, with that of the comparable sample. In our experiments the value for K_{CO_2} was calculated using $\alpha_{CO_2}=0{\cdot}82$. This is the solubility of carbon dioxide in water at 22·5° C. It is taken that this is not appreciably affected by glucose, and we have assumed that the solubility in the living tissue is the same as that in water, and that there is no appreciable retention of CO_2 by the tissue proteins.

It is doubtful whether the method of Dickens & Šimer has any advantage over that of Warburg, when, as in these experiments, it is possible to obtain comparable samples. In Table II are set out the results of those experiments in which both methods were used on similar material. The differences between the values obtained are very little outside the limits of experimental error except in Exp. 22, in which low partial pressures of oxygen were used.

TABLE II. *Comparison of two methods*

			R.Q.	
Exp.	Gas	Medium	D. and Š.	Warburg
M 16	100 % O_2	2·5 % glucose, (iodoacetate)	1·06 1·08	1·04 1·04
M 17	100 % O_2	2·5 % glucose, (iodoacetate)	0·99	1·00
M 14	5 % O_2	2·5 % glucose	1·64	1·63
M 22	1 % O_2	2·5 % glucose	4·8	5·3

(*e*) *Measurement of fermentation rate.*

It is possible to measure rates of fermentation in the manometric apparatus by passing pure nitrogen into the vessel and measuring the increase in pressure due to carbon dioxide output. Two disadvantages attend this method. First, the CO_2 accumulates over the tissue, and unless it is swept away at intervals by the passage of fresh nitrogen, the experiments cannot be prolonged. In an average experiment the concentration of CO_2 in the vessel, after 5 hr., is about 3 %; higher partial pressures of the gas may affect the fermentation rate. Secondly

it is difficult to measure exactly the extent to which CO_2 evolved is bound by the living tissue, and unless we do know this, exact values for carbon dioxide output cannot be obtained. For comparative purposes this error is unimportant. We usually have assumed that there is no retention of CO_2, and that the solubility of CO_2 in the tissue is the same as that in water. This assumption is also made in calculating the R.Q. in the direct method, and the similarity of values obtained by this method, and that of Dickens & Šimer, suggest that no large error is introduced in this way.

It was sometimes necessary to measure the fermentation manometrically, immediately following a period of respiration estimations. A special vessel designed for the purpose not being available, the following procedure was adopted. The alkali used during the measurement of respiration was placed in the side bulb, with filter paper. Before the transition to nitrogen, the alkali and filter paper were removed, and the side bulb and annulus thoroughly washed out with acid. The vessel was then replaced on the manometer, and pure nitrogen was passed through it. Fermentation readings could then be obtained about half an hour after the last respiration reading. Nitrogen was usually passed from the furnace at the rate of 5–7 l. an hour, and escaped through the loosened side tap. The vessels were meanwhile supported on a frame outside the bath, and were gently shaken from time to time. They were shaken in the bath for 10 min. before the first reading was taken.

(*f*) *Limiting thickness of the tissue slice.*

The slices used in all manometric experiments were cut 0·1 cm. thick. It may be shown that when slices are placed in water in equilibrium with air, the cells at the centre of each slice receive oxygen by diffusion through the slice at such a rate that the oxygen concentration in these cells is above the "extinction point".[1] In other words these cells respire and do not carry out fermentation at an appreciable rate.

For the determination of the limiting thickness of the tissue slice, i.e. the thickness at which the internal oxygen concentration is zero, Warburg applied the formula given on p. 50 of Dixon's book (1934). Applying this to our own material, and taking a maximal value for the respiration rate, we find the minimum thickness permissible in respiration experiments, to be 0·3 cm. It is, however, better to test

[1] A phrase used by F. F. Blackman to indicate that concentration of oxygen above which fermentation ceases.

this empirically, and this has been done. The following results show that adequate aeration at the centre is obtained by the use of slices 0·1 cm. thick.

(α) The R.Q. of slices 0·1 cm. thick, in water equilibrated with air, was not significantly different from that of similar slices in water equilibrated with pure oxygen (Table III).

TABLE III

Exp.	Gas	Hours washed	R.Q. (D. and Š.)
M 12 V	100% O_2	14	0·97
M 12 III	Air	14	0·98
M 12 IV	Air	14	1·00

(β) Although on transference of slices from water in equilibrium with air, to that in equilibrium with oxygen, one sometimes found an increase in the rate of respiration, the increased rate was only slowly attained. There was not an immediate rise to a new steady value, such as one obtained on transferring tissue from 2·5% oxygen to air, and which one would expect to find if the inner cells of a disk were oxygen-starved (see Graph M 12, Fig. 4).

(γ) The R.Q. of slices only 0·05 cm. thick was not significantly different from that of slices of the standard thickness (Table IV).

TABLE IV

Exp.	Gas	Thickness of slice cm.	Hours washed	R.Q. (D. and Š.)
M 30 II	Air	0·1	45	1·08
M 30 IV	Air	0·05	45	1·08
M 30 V	Air	0·05	45	1·08

(δ) There was no significant difference between the R.Q. of slices freshly cut and respiring rapidly, and that of slices whose respiration rate had declined to about 50% of the initial high value. The supply of oxygen to the internal cells is therefore adequate to allow respiration, even when the outer cells of a slice are rapidly absorbing oxygen.

In manometric experiments the ratio of tissue to medium is usually 1 g. per 3 c.c. and the liquid is shaken in contact with the appropriate gas mixture. In the Pettenkofer experiments the ratio is of the same order (1 g. per 4·4 c.c.), and the gas mixture is passed through the liquid in a stream of fine bubbles. We take it therefore that the slices are under comparable conditions in the two types of apparatus. It seems very probable therefore, from the above results,

that the rate of carbon dioxide production, measured in the Pettenkofer apparatus, is a true index of respiration rate, so long as the slices are 0·1 cm. thick. Some experiments of Mr El Gawadi (unpublished) confirm this conclusion, for it was shown that alcohol production, by slices under the conditions of the Pettenkofer experiments, was negligible.

In some experiments with both types of apparatus, the tissue slices were placed in glucose solution, and subsequently showed a high rate of respiration. There was some evidence that when these solutions were equilibrated with air, some fermentation accompanied the respiration. The R.Q. was considerably above 1·00, and on passing pure oxygen, the respiration rate rose immediately to a new high steady value, while the R.Q. declined to about 1·1 (Table V).

TABLE V

Exp.	Solution	Gas	R.Q.	Respiration rate
M 13 VI	5% Glucose	100% O_2	1·09	58
M 13 V	5% Glucose	Air	1·22	41

The high R.Q. is taken to show that some fermentation is going on in the slices in glucose equilibrated with air, which is not entirely eliminated when pure oxygen is passed. It is possible that this fermentation is carried out by the surface cells in immediate contact with the glucose solution; or it may be restricted to the internal cells. Experiments with slices of different thicknesses are planned to elucidate this point. There is no doubt however, in those Pettenkofer experiments in which glucose solutions were used, that the rate of CO_2 output was not a true index of the respiration rate. In these experiments it would have been better to have passed oxygen, instead of air, through the vessels, or possibly the same purpose would have been served by the use of slices less than 1 mm. thick.

V. PRESENTATION OF RESULTS

In Figs. 3 and 4 we plot the results of typical experiments with paired samples of carrot slices. The curve of Fig. 4 was given by a Pettenkofer experiment, and shows the drift of respiration with time, and the effect of nitrogen on the carbon dioxide output of the tissue. Fig. 5 gives the result of a manometric experiment, in which four samples were used. They were all in water, equilibrated with air, oxygen and 5% oxygen respectively. The method of working out the R.Q. is given in Appendix II.

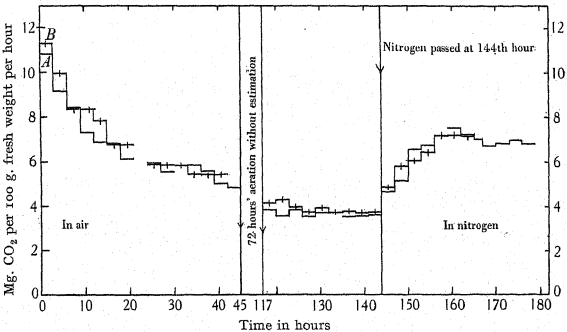

Fig. 3. Exp. M 16. Respiration rate of carrot slices, as measured by the continuous current apparatus. Slices were washed for 190 hr. before the experiment began. *A*, 43 g. fresh weight; *B*, 41 g. fresh weight; both in 200 c.c. of distilled water. Temp. 22·5° C.

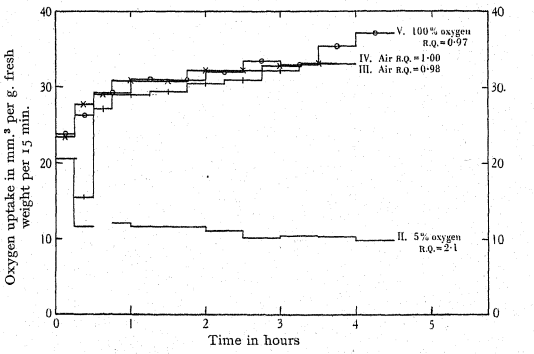

Fig. 4. Exp. M 12. Respiration rate and R.Q. of carrot slices as measured by the manometric method. Slices were washed for 14 hr. before the experiment began; the gases were passed for 25 min. before the first reading. Temp. 22·5° C. The slices were suspended in distilled water.

APPENDIX I. NOTES ON THE ANATOMY OF THE CARROT SLICES USED IN THE EXPERIMENTS

The carrot slices are largely composed of a secondary parenchyma, the cells of which lie in regular rows, having been formed by tangential divisions in a well-marked cambium. Typical large cells are illustrated in Fig. 6A, B, and smaller ones in Fig. 6F. The larger cells are usually separated at the corners by air spaces, and all the parenchyma cells are vacuolated. Large and small, irregular, or crystal-shaped plastids containing carotin lie in the peripheral protoplasm. The nuclei are small, and are not obvious without staining.

Morphologically the parenchyma may be classified as xylem and phloem parenchyma, and in most slices the two kinds are present in about equal amounts. In some of the large and irregular slices used in the Pettenkofer experiments, there is also included parenchyma of the pith and cortex, which shows a non-radial arrangement of the cells.

In Fig. 5A, B, we represent the position and extent of the various tissues in a slice of the type used in manometric experiments. In addition to the parenchyma which forms the bulk of the slice, there are the following tissues:

(*a*) *The cambium* (Figs. 5; 6C, E; 7G). In most slices this runs across the diameter. It has not been observed to add new cells to the stored roots or to tissue slices in aqueous solutions.

(*b*) *The xylem* (Figs. 5; 6C, D; 7J). This consists of small bundles of scalariform tracheae distributed as shown in Fig. 5. They do not always run vertically, and are often oblique in a transverse section. As shown in Fig. 6D, the parenchyma cells around those bundles laid down early in cambial activity, have divided tangentially to the bundles, so that their originally radial arrangement is obscured.

(*c*) *Small xylem parenchyma cells* associated with the xylem bundles (Fig. 6C), but not otherwise markedly different from the rest of the xylem parenchyma. For instance they do not differ in staining capacity, and they lack starch.

(*d*) *Medullary rays* (Figs. 5, 6B). These are one to several cells wide in transverse section, and often many cells deep, and they run radially through the secondary tissues. They are distinguished from the parenchyma by the greater length and smaller width of their cells, by their greater capacity for the uptake of aniline-blue, and sometimes by the possession of a greater amount of carotin. The cells of the rays in the xylem do not differ from those in the phloem.

(*e*) *The phloem.* On a first examination this is not obvious: it consists of very narrow sieve tubes and companion cells (Fig. 7G). They are to be seen in those radial longitudinal sections which have passed through a xylem bundle. In transverse sections the phloem is identified as very small groups of tiny cells lying opposite the xylem bundles (Figs. 5, 6F). It is difficult to determine how much true phloem is present in the wide belt of secondary tissue external to the cambium. Occasionally, longitudinal sections show old, possibly non-functional sieve tubes lying in the periphery of the secondary parenchyma.

Probably the dead xylem is roughly equivalent in amount to the living phloem, and together they make up about 2% of the total volume of tissue in a slice.

(*f*) In some of the slices used in manometric experiments, and in the larger ones used in Pettenkofer experiments, there must also be included some secretory tissue (Fig. 1), which surrounds minute canals lying in the outer phloem parenchyma. As in other Umbelliferae, these canals are schizogenous, and the cells around them are often stretched tangentially. These cells do not differ

much from the other living cells in appearance; in fact they are most obvious as streaks under the $\frac{2}{3}$ in. objective It is presumed that they contain, or secrete, substances which help to give the characteristic flavour to the carrot root, and they may differ in respiratory metabolism from the other cells. They are however present in negligible quantities in all slices.

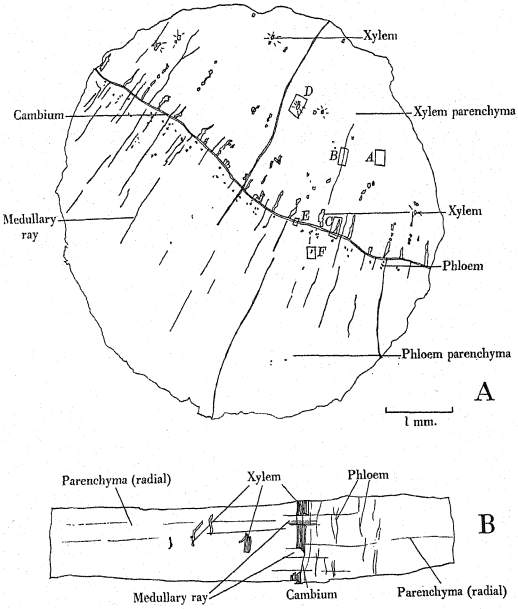

Fig. 5. Carrot tissue slice, 6·3 mm. diameter, 1 mm. thick as used in manometric experiments. A. Transverse section. B. Longitudinal section.

Up to the present no attempt has been made to distinguish physiologically between the various tissues described, except that it has been shown that, in some roots at least, the phloem parenchyma is richer in peroxidase than is the inner tissue. "Oxygenase" is apparently limited to the outer layers of the primary cortex, in cells not included in the experimental material.

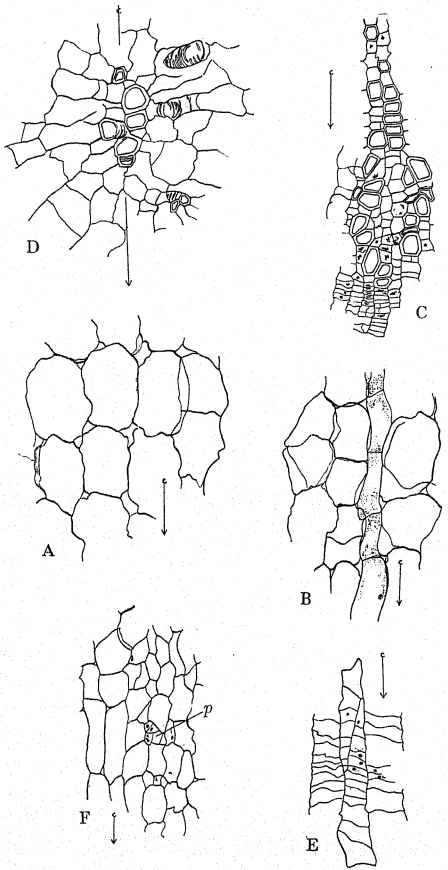

Fig. 6. High power drawings of carrot tissues from positions shown in Fig. 5A. (Transverse section $\times 380$.) C, D, xylem; A, xylem parenchyma; B, medullary ray in xylem; E, cambium; F, phloem. The arrows are radial and all point to the periphery.

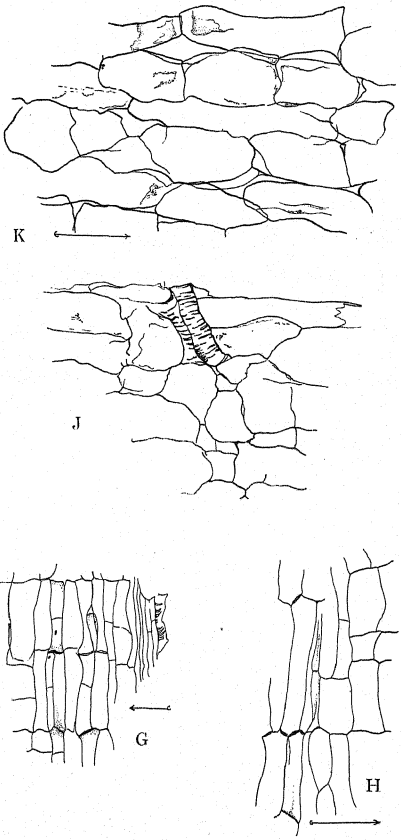

Fig. 7. High-power drawings of carrot tissues. (Longitudinal section ×400.) J, xylem vessel in parenchyma; K, phloem parenchyma; G, phloem and cambium; H, phloem. The arrows are radial and point to the periphery.

APPENDIX II. TYPICAL PROTOCOL OF MANOMETRIC EXPERIMENT

Exp. M2. 4 *Nov.* 1932. Total duration of experiment 130 min. Temp. 22·5° C. Slices washed for 26 hr. after cutting.

Vessel No.	V	III	I
Total vol.	26·09 cc.	26·85 c.c.	25·42 c.c.
Side bulb	0·3 c.c. 2·5*N* HCl	0·3 c.c. 2·5*N* HCl	0·3 c.c. 2·5*N* HCl
Main	2·5 c.c. H_2O	2·5 c.c. H_2O	2·5 c.c. H_2O
Tissue	None	25 slices	25 slices
Vol. tissue		1·26 c.c.	1·26 c.c.
Fresh weight tissue		1·18 g.	1·25 g.
Annular	0·4 c.c. baryta	0·4 c.c. baryta	0·4 c.c. baryta
Gas	100% oxygen	100% oxygen	100% oxygen
V_g (vol. gas)	22·89 c.c.	22·39 c.c.	20·96 c.c.
V_f (vol. liquid)	3·2 c.c.	4·46 c.c.	4·46 c.c.
First reading, at 1.50 p.m.	14·38/15·00	22·90/25·00	23·33/25·00
		Tipped acid	

	(Reading	Diff.	Corrected)	(Reading	Diff.	Corrected)	(Reading	Diff.	Corrected mm³ O_2)
+15 min.	14·38	0·00	0·00	25·23	+2·33	+2·33	21·79	−1·54	−1·54	30·1
,,	14·56	+0·18	0·00	Acid tipped again			19·56	−2·23	−2·41	47·0
,,	14·89	+0·33	0·00	25·58	+0·35	+0·17	17·53	−2·03	−2·36	46·0
,,	14·90	+0·01	0·00	25·97	+0·39	+0·06	14·98	−2·55	−2·56	49·9
,,	15·19	+0·29	0·00	25·98	+0·01	+0·00	12·80	−2·18	−2·47	48·2
,,	15·03	−0·16	0·00	26·19	+0·21	−0·08	10·07	−2·73	−2·57	50·2
,,	15·10	+0·07	0·00	25·99	−0·20	−0·04	7·59	−2·48	−2·55	49·7
,,	15·38	+0·28	0·00	26·12	+0·13	+0·06	5·30	−2·29	−2·57	50·2
,,	15·59	+0·21	0·00	26·39	+0·27	−0·01	2·89	−2·41	−2·62	51·1
,,		Acid tipped		26·58	+0·19	−0·02		Acid tipped		
,,	15·28	−0·31	−0·23	26·50	−0·08	0·00	23·78	+20·89	+20·97	—
,,		Tipped again						Tipped again		
,,	15·68	+0·40	+0·55	26·35	−0·15	0·00	23·67	−0·11	+0·04	—
,,	15·89	+0·21	−0·01	26·57	+0·22	0·00	23·88	+0·21	+0·00	—
h O_2		—			—			−216·5 mm.		
k O_2		—			—			1·95		
Total oxygen uptake in mm³								422·3 mm³		
h CO_2		3·1 mm.			24·7 mm.			210·1 mm.		
k CO_2		2·37			2·41			2·28		
Total CO_2		7·35 mm³			59·6 mm³			479 mm³		
Solution correction		0·00			52·25 mm³			471·7 mm³		
Tissue correction		—			0·00			55·3 mm³		
Total corrected CO_2 output in mm³								416·4 mm³		

$$\text{R.Q.} = \frac{CO_2}{O_2} = \frac{416{\cdot}4}{422{\cdot}3} = 0{\cdot}99.$$

The last column on the right under I gives the rate of oxygen uptake per 1·25 g. per 15 min.

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STUDIES IN THE AUTECOLOGY OF *CLADIUM MARISCUS* R.BR.

IV. GROWTH RATES OF THE LEAVES

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(With 16 figures in the text)

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INTRODUCTION

It was noticed by Dr Godwin during his ecological investigation of Wicken Fen, that *Cladium Mariscus* shows an unusual feature in that the inner, younger, leaves of each shoot all grow upwards at the same rate. Thus, if a crop of sedge is taken so that all the leaves are cut off at the same level close to the ground, those of the inner group keep level for a long time, and, months later, their cut ends will still be within a millimetre of one another. It was therefore suggested to the writer that the matter would be worth further study. Little if any attention seems to have been given to this type of growth behaviour in the literature on monocotyledons, and no systematic search has yet been made to find out whether it is a widespread phenomenon in the Cyperaceae, or other families. It is known to be exhibited, however, by *Carex riparia* and *C. elata*.

A description of the anatomy of the meristematic regions of the leaves and the growing point of the stem has already been given in the first paper of this series (Conway, 1936*a*) and a qualitative account was also given of the phases of growth and development through which each leaf passes. The present paper deals with quantitative measurements of the rate of elongation of the type B leaves, that is, of those leaves with a meristematic region at the base which includes

a zone of cell extension and which is traversed by one or more provascular strands. This upward growth of the leaves will be referred to as *extension* though it is not intended to involve any assumption as to the physiological activities by which it is brought about. The word *growth* will be used as a general term.

The measurements of extension rates were begun with a view to exploring the physiological behaviour of the meristematic region in the species, but it soon became apparent that the results might be of great interest to the field botanist, because the impress of environmental factors can be so clearly seen in the fluctuations of extension rate. The data are dealt with here entirely from the ecological standpoint, since their physiological interpretation, though of great interest, does not lie strictly within the scope of this series of papers. To discuss the results in terms of the plant metabolism would take up much space, and cannot in any case be done satisfactorily without further experimental investigations.

Weekly measurements of extension

Observations were made both on plants growing in Wicken Fen and on others which were transplanted from the Fen and established in experimental conditions in the Botanical Laboratory at Cambridge. The transplant experiments were mainly confined to the year 1935, but records of the same set of Fen plants were kept throughout that year and on through the summer of 1936. The marked contrast between the hot summer of 1935 and the cool wet summer of 1936 has given added interest to the results.

The method of measurement for the Fen plants was to mark all the type B leaves at the same level with a horizontal line in Indian ink. A small wooden platform was pegged firmly into the ground beside the base of each plant and this served as a base on which to stand a metre scale for measuring the height of the ink marks. The first set of marks were usually made at 30 or 50 cm. above the platform, according to convenience, and when they had been carried up more than 20 cm., new marks were made again at 30 or 50 cm. Height readings were made at weekly intervals as a rule, though sometimes in the winter a fortnight was allowed to pass between readings.

Six plants were chosen in the Fen, two in each of the three plant communities in which *Cladium* is abundant, namely Pure Sedge (Cladietum), Mixed Sedge (Cladio-Molinietum) and, in its earlier

stages, Carr (Rhamnetum frangulae) (cf. Godwin, 1931, 1936; Godwin & Bharucha, 1932). The Pure Sedge community is scantily represented at Wicken, and consists mainly of the vegetation occupying the margins of a series of long narrow ditches. The two plants investigated in this region, E and F, were growing a few yards away from each other, both at the side of a ditch and at a lower soil level than the plants A and C, chosen in the Mixed Sedge community. The ecological differences between the two habitats lie firstly in the different soil level and hence different relation to the soil water-table and secondly in the four-yearly crop cutting which affects the Mixed Sedge but not the Pure Sedge. The other pair of plants, G and H, were situated just inside a patch of young Carr adjoining the Pure Sedge region; the distance between this pair, and E and F was not more than 10 yards. As far as soil and water-level relations are concerned, conditions for G and H were very similar to those for A and C and the habitat difference lies in the complex of conditions introduced by the tree layer. Plants A and C were within a few yards of the thermograph and water-level recorder from which the environmental data have been derived; the other plants were about 200 yards away, so that the data should apply to them also without serious error.

The plants used in the laboratory experiments were placed in groups of five or six in fen-peat contained in teak boxes 30 cm. square and 30 cm. deep. The latter were pierced through the bottom to allow water to enter, and were placed in metal containers 15 cm. deep, so that it was possible if desired to saturate the lower 15 cm. of the peat, in which the plant roots were situated. The method of measuring extension by means of horizontal Indian ink marks was used here again, but the fixed base was provided by a lath of wood laid across the top of the box.

The plants were transplanted early in March 1935 and all kept together on the roof of the Botany School, in a place which gave protection from wind, and they were all fully watered, i.e. the outer metal containers were kept full of water. On 23 April three sets were taken and placed under the conditions of the experiment. One set (plants H 1 to 6) were placed in a greenhouse on the roof and kept fully watered, in order to show the effect of increased temperatures, given adequate water supply. The second set (D 1 to 6) were placed out of doors on the roof but underneath a glass shelter to keep off the rain. No more water was added to the container which became dry after a week; after this, a measured quantity of water was added to the peat surface every day. For the first fortnight

50 c.c. a day was added, but as the plants appeared very unhealthy (many of the green leaves withered in their upper parts) the amount was increased to 100 c.c. This still seemed insufficient and 200 c.c. a day were given till the end of July, when the amount was reduced to 100 c.c. During September the set was given a full water supply. All the plants except D 6 appeared very unhealthy, and two of them died. The results for D 6 were, however, interesting and are discussed later. The third set was kept out of doors on the roof, sheltered from North and East winds (to avoid mechanical damage), and kept fully watered throughout the summer.

The results for all the Fen plants are given in Figs. 1 and 3, and for selected plants of the experimental set in Fig. 1. These figures give certain environmental data as well. At the top are shown the daily maximum temperatures recorded on the uppermost bulb of the thermograph which was used also in investigating soil temperatures (Conway, 1936*b*). This bulb was placed in the shade of the Mixed Sedge vegetation, 5 cm. above the soil surface. Its readings may be taken to give a general picture of weather conditions, especially when considered together with the rainfall data, and hence may be used also in comparison with the extension-rate curves for the plants which were out of doors in Cambridge. The rainfall data have been plotted from the daily rainfall records at Fordham, a village about four miles away from Wicken. These data were very kindly supplied to me by Mr W. V. Bloom of Fordham. The level of the water-table in the middle of the Fen is also shown in the figures and has been plotted from the permanent water-level recorder situated close to plants A and C.

Fig. 2 shows the results for the extension rates of all the plants C 1 to 5, and has been given in order to show the degree of agreement between plants growing under the same conditions. It will be seen that there is a good agreement between all of them with regard to the times of pronounced maxima and minima, and further that the general shape of the curve, considered over the whole season, is very similar for all. The only outstanding difference lies in the lower general level of the curve for C 5. On inspecting the plants it was clear that this plant differed from the other four in having fewer, and rather shorter leaves—it had in fact, a less healthy appearance. Hence it seems reasonable to conclude that if a plant resembles in appearance the other plants which grow in a similar set of conditions, its extension-rate curve may be taken as representative for that particular plant community.

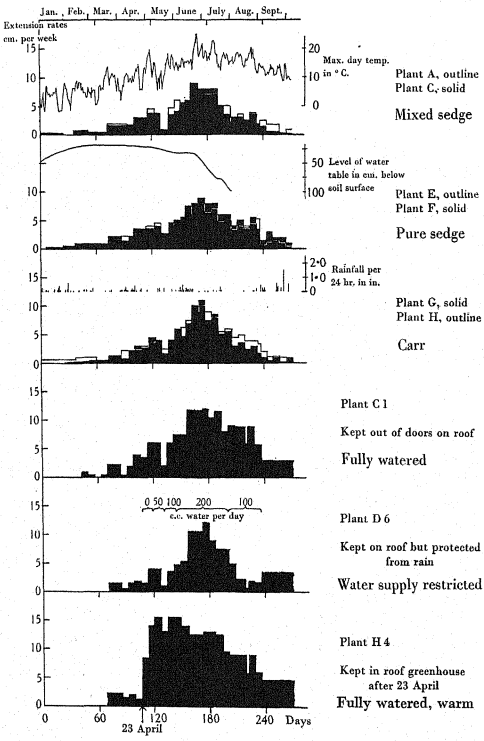

Fig. 1. Results of weekly extension measurements of plants on the Fen and under laboratory conditions, from January to October 1935.

This conclusion is borne out by the agreement between the two members of each pair of Fen plants. On the whole they agree more closely with one another than they do with those of the other pairs, a point which is quite striking when one considers that the climatic

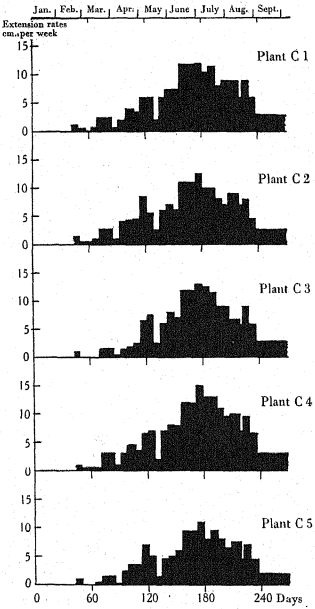

Fig. 2. Extension rates of five plants kept under the same conditions during the summer of 1935.

conditions are exactly the same for all, so that it is just the local conditions which are inducing the differences.

These differences between the Fen plants are concerned more with the general shape of the curves than with the fluctuations from week to week. The correspondence in the latter which is shown by

all the curves is very remarkable and it can be seen from Figs. 1 and 3 that they are closely correlated with the changes in temperature. The most conspicuous feature is the very sharp drop in extension rate which is apparent in all the curves in the middle of May 1935, and was undoubtedly caused by the severe night frosts which occurred for several nights running at that time. Its influence is shown even in the case of plant H 4, which was growing in the greenhouse, and so not exposed to actual frost, but only to relatively lower temperatures.

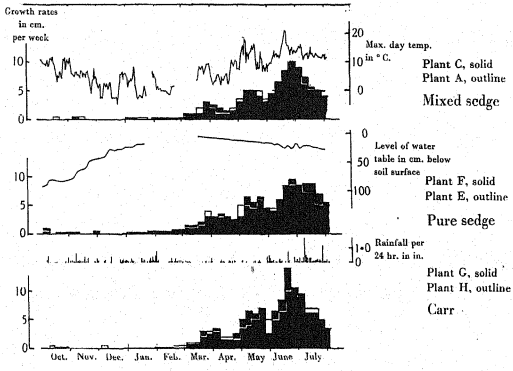

Fig. 3. Results of weekly extension measurements of plants on the Fen from October 1935 till August 1936.

From the ecological point of view, considerable importance attaches to the differences in the general drifts of the graphs in Fig. 1, on which are superposed the minor fluctuations just discussed.

If the graphs for plants A and C are compared carefully with those for E and F, it may be seen that the latter conform more closely to the general course of the temperature curve, in that during the last half of July and August (from about the 200th to the 240th day) the extension rate is nearer to its maximum value. The temperatures during this period are not strikingly lower than the maximum values, and do not show the strong fall from the maximum that is exhibited by the extension-rate curve for A and C.

A possible explanation of this fact may be derived from the curve showing the soil water-level, which by the end of July is more than 100 cm. below the surface. The absolute water-levels in the two situations are not likely to be very different, but since the Pure Sedge plants E and F are rooted 30 or 40 cm. lower than A and C, it is quite possible that the latter were suffering from a deficit of soil water as compared with E and F. This suggestion is strongly confirmed by the graph for plant C 1, whose roots were growing in waterlogged soil throughout the summer. Here the extension-rate values in August are still closer to the maximum, roughly two-thirds of it, as compared with about half in the case of E and F, and about a third for A and C. For this reason the curve for plant C 1 is more closely correlated with the general temperature drift, and the idea is confirmed that soil water supply is acting as a limiting factor on the extension rates of the Fen plants during the late summer. It is interesting to note that the peat was damp to the touch all over the Fen; the species therefore seems definitely to demand a soil not merely moist, but actually waterlogged.

The effects of diminishing the water supply artificially are shown by the results for plant D 6. The extension rates are much lower than the control plant C 1 except over the period when 200 c.c. of water were being given. This quantity appears to have been sufficient to allow the maximum growth rate, taking plants C 1 to 5 as a standard, and though the effect was produced unintentionally, it is interesting as it shows the response of the extension rates to higher temperatures, given adequate water. Similarly in September the extension rate is as high as for the controls, but the rest of the curve exhibits the severe limitation induced by water shortage.

The curve for H 4, which is quite typical of all the plants H 1 to 5, shows that an increase in temperature can raise the extension rate early in the growing season, as well as later on; in other words, that the seasonal growth cycle shown by the plants in the field is induced by the natural climatic cycle. No temperature readings were taken in the greenhouse, but it was hotter inside it during May and June than later, because the roof was whitewashed later. The experiment was confirmed in 1936, taking temperature readings, and the results are given in Fig. 4. Two plants were grown in pots of peat in the greenhouse and two others on the roof just outside; the peat in both cases was kept waterlogged. Fig. 4 shows the extension-rate curves for one out of each pair, and the mean maximum day temperature for the week, taken close to the experimental

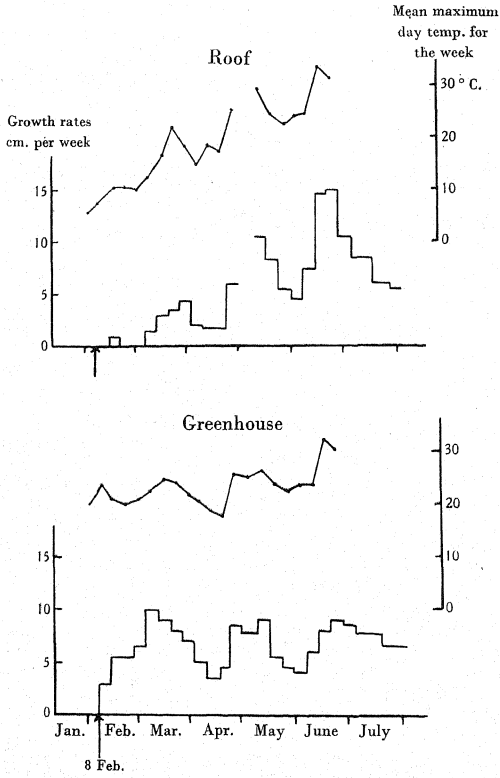

Fig. 4. Results of an experiment to demonstrate the correlation between temperature and extension rate.

plants. The way in which the artificial temperature conditions have removed any signs of a midsummer growth maximum is clearly shown.

It is worth noting in the curve for the greenhouse plant in Fig. 4, that though the temperatures in June are higher than those in May, the extension rates are slightly lower. It seems likely that this is because the light in the greenhouse is more diffuse than that out of doors, especially after the roof has been whitewashed in early June, though the reasons for this view will be discussed later.

During the whole of 1936 the water table all over the Fen remained within 25 cm. of the soil surface, when it was not actually above it, so that the roots of the *Cladium* plants were continuously in water-logged peat. There is a close correlation between the general drifts of the extension-rate curves and the temperature curves until the beginning of July. During July the extension rates appear to fall more rapidly than would be accounted for by the fall in the temperature curve. Such a divergence was seen in the curves for plants A and C during 1935, but in 1936 it cannot be accounted for by lack of soil water. On the contrary, it can be explained by an examination of the rainfall data in Fig. 3, which indicate that though maximum day temperatures may not have fallen below 10° C., the weather was for the most part dull and wet.

Comparison of Figs. 1 and 3 shows further that although the temperatures in 1936 are on the whole much lower than those in 1935, the total extension, as measured by the area under the curves, is much the same for both years, suggesting again that water supply was insufficient in the field conditions in 1935.

No attention has yet been paid to the results for plants G and H, growing in Carr. There is a slight difference between the two which is attributed to the situation of H in a less shaded place. It is therefore not surprising that the graph for H more nearly resembles those for A and C in the Mixed Sedge, than does the graph for G. The soil conditions are very similar in Carr and Mixed Sedge.

The two features in the curve for G which are of special interest are shown both in 1935 and 1936. In the first place it shows a larger range of fluctuation in response to the minor variations of temperature than other curves, and secondly it is more concave on either side of the maximum value than any of the other curves. These characteristics may indicate either a change in the reaction of the plant to a given temperature variation, or a difference in the actual temperature conditions within the Carr and outside it, or possibly both together.

Some data which elucidate this point were obtained from the continuous records of growth and will be described later.

It is clear from Figs. 1–4 that in addition to variations in shape of the curves there are differences in general pitch, which imply great differences in the total increase in leaf area. These can be caused by altering the external factors, as may be seen for instance in the contrast between plant C 1 and plant A, and also by initial differences in plants in the same conditions, as discussed already in connection with Fig. 2. Moreover, when the pitch is higher not only does each leaf elongate more rapidly, but more new leaves are produced. Thus, during 1935, plant C 1 produced 8 new type B leaves, the corresponding number passing over into the type C stage (no longer growing actively), whereas the number for plant A was only 5. Since there is this correlation between the luxuriance of the plant and the magnitude of the extension rates, this is an added reason for considering that the extension rate is useful as an ecological indicator for the species.

The main points arising from the weekly growth measurements are as follows:

(1) There is a high degree of consistency in behaviour between similar plants growing in similar habitats, so that it is possible to draw general conclusions from results obtained with a small number of plants.

(2) Although at first sight the differences between the various *Cladium* habitats on the Fen appears small, they are important enough to affect the behaviour of the plant as exhibited in the variations of extension rate through the season.

(3) The two factors of day temperature and soil water supply show their effects very directly on the extension rate; while qualitatively the extension rate always shows a positive correlation with temperature, its quantitative response to temperature change is greatly affected by the available water supply.

CONTINUOUS RECORDS OF EXTENSION

In order to find out in more detail how the extension rate varies from day to day as well as from week to week, it was decided to set up self-recording auxanometers in the field. The species is one which lends itself to the use of auxanometers because all the growth that is being measured takes place in the same vertical direction, and because it is so large, sometimes a centimetre or more in a day, that

it is not necessary to have delicate apparatus which would require constant attention. The auxanometers were planned in principle by Dr Godwin, and designed in detail and constructed by Messrs Negretti and Zambra.

The main features of the apparatus are shown in Fig. 5. The lever arm *AB* is attached near one end to one of the type B leaves of the plant. The other end can be locked by means of the screw *C* to the upper shaft *DE*, which passes through the lever arm. When the leaf moves upwards, the rotation in the shaft *DE* is transmitted to the lower shaft, which carries the pen arm. The movement is transmitted through the link *HJ*, which joins the two bars *KL* and *MN*. These two bars can be pushed through the blocks *P* and *Q* to the desired extent and fixed by the screws *R* and *S*. By adjusting their positions it is possible to arrange whether the actual height increase shall be magnified or reduced on the record sheet. The aim is, of course, to obtain as much magnification as possible without causing the pen travel to exceed the height of the record sheet. The scale of the record can be varied from a magnification of 3 to a reduction of $\frac{1}{2}$ on the real height increase. It is necessary to employ the reduced scale when the growth rates are high in midsummer.

The method of attachment to the plant is also indicated in Fig. 5 (iii). The last 15 cm. of the lever arm is pierced at intervals by 2 mm. holes. A small brass cylinder, about 1 cm. long and 5 mm. in diameter is soldered to a metal strip which is attached to the lever arm by a nut and bolt passing through one of the holes. The leaf passes through the cylinder and is held firmly by a cork which fits the cylinder but which is cut into two pieces which fit round and into the V-shaped leaf lamina. They are applied to the leaf and the brass cylinder is then pushed down firmly on to them so that the leaf is gripped but does not suffer any injury. There has never been any indication that this method of attachment harms the leaf in any way. The position of the cylinder on the leaf is altered when necessary so that the end of the lever arm shall always travel within the limits of the same arc, that is, between a height on a level with the upper shaft *DE* and a height of 10 cm. above this.

The lever arm is slightly flexible so that when a strong wind blows against the plant to which it is attached it oscillates from side to side and this would cause considerable disturbance in the record if an arrangement were not made to avoid the wind action. The far end of the lever arm which projects beyond the leaf passes through a space between two wires of a framework which is clamped

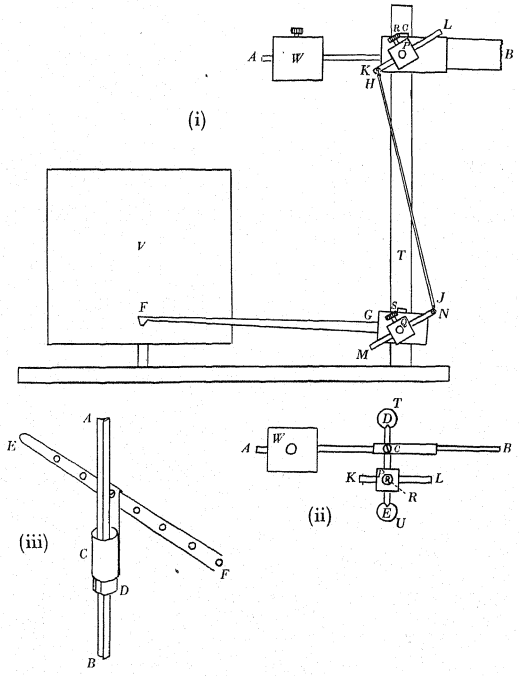

Fig. 5. Construction of auxanometer. (i) Elevation. (ii) Plan of upper part of apparatus. AB, lever arm; C, screw which locks AB to the horizontal shaft DE; FG, pen arm; HJ, link connecting the rods KL and MN which pass through the blocks P and Q respectively and are locked by the screws R and S; T and U, upright supports; V, recording drum; W, balance weight. (iii) Method of attachment to plant leaf. AB, leaf; C, brass cylinder; D, pieces of cork fitting round the leaf; EF, distal end of lever arm.

in a suitable position. This framework is a wooden rectangle 20 cm. high and 10 cm. wide. Parallel wires are stretched along the length of it, and are about 7 mm. apart from one another. The framework is arranged so that the wires are vertical and the end of the lever arm can move up between two of the wires without touching either when it is in a normal position. Should it be affected by the wind, however, a large sideways movement is prevented by the presence of the wires.

This framework is used also to serve another purpose. The lower horizontal bar of the rectangle carries a stiff wire which projects horizontally at right angles to the plane of the frame and thus runs parallel to the lever arm but below it. This wire forms a permanent base from which it is possible to measure the height of the brass cylinder. If this height is measured at the beginning and end of the week the difference is the true height increase of the leaf during the week and this quantity can be used to calibrate the readings given by the record sheets.

Each auxanometer was fixed in position on a firm platform, supported on stout oak stakes driven into the ground near the plant which had been selected. Another stake was driven in on the far side of the plant to carry the framework of wires just described.

Four auxanometers were obtained and were set up in the field early in June 1935. Two pairs of plants were chosen for investigation, namely, P and Q in the Pure Sedge, and R and S in Mixed Sedge. Each pair was growing quite close to the corresponding plants E and F, A and C for which the extension-rate data have already been described. They were all chosen to be as like each other as possible in height and general appearance. Records were kept of all four up to May 1936 except for a period of some weeks in the early part of that year when floods made it necessary to remove the apparatus. From May to August 1936, plant S was left, and the instrument was moved to a plant T which was growing in a shaded position in the region of Carr close to the plants G and H.

Fig. 6 has been prepared from the records for the week of 25 June to 2 July 1935. This was a week of almost uniformly fine weather, the third day of the week, 27 June, being the only exception in having considerable periods of overclouding, possibly even showers. The extension curves are typical of such fine weather and though the scale is reduced in Fig. 6, it is possible to make out certain general features.

In the first place there is a very close agreement in behaviour

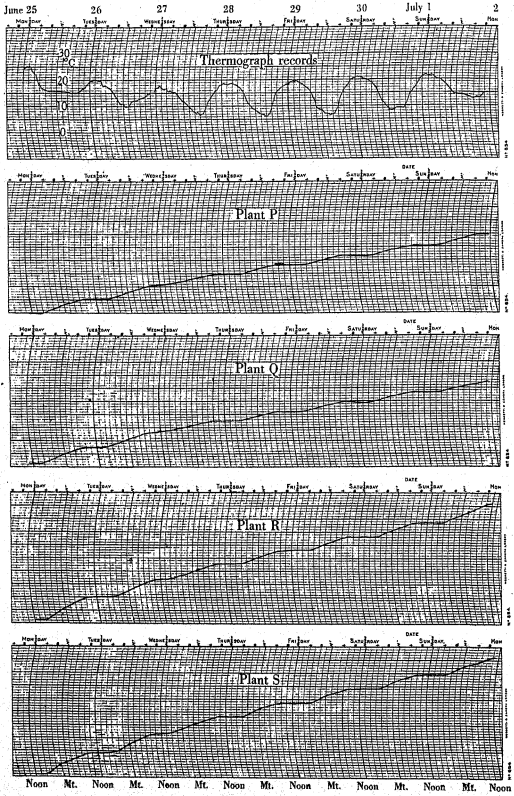

Fig. 6. Tracings of the records for all four auxanometers from 25 June to 2 July 1935, with shade temperatures 5 cm. above soil surface.

between the two plants of each pair, and there is a considerable similarity between the two pairs. It is clear that extension is entirely prevented for some hours on either side of noon, except on the day when there was overclouding, as indicated by the type of temperature record. On this day, though the extension rate is diminished, it is not reduced to zero. The details of the curves in Fig. 6 need not be considered, since slight irregularities may have entered through the technique which had to be used to make the figure. The characteristics of the typical curves for 24 hr. may be illustrated by the more accurate reproductions of selected sections of the records, given in Figs. 7–13.

The type of weather which prevailed on any given day can be judged fairly accurately from the temperature records obtained from the thermograph, and whenever they indicate clear warm weather, the extension curves show certain typical features. The first of these is the very sharp change over from a rapid extension rate to a zero rate in the morning and the converse change in the evening. This is illustrated by the curve for plant R on 28 June 1935, shown in Fig. 7. The length of time for this transition is not more than $\frac{1}{4}$ hr., which seems remarkably rapid. Fig. 8 is the curve for R on 10 August and shows a feature which is fairly common in all the records during the hottest weather, namely a slight negative extension rate, or shrinkage during the day, which is attributed to excessive loss of water by transpiration from the leaf cells. Both these records indicate that the extension rate is almost constant during the whole night's growing period, but it is often found in the records that the rate is higher initially and then falls to a steady value which continues through the rest of the night. Plant S showed this characteristic more strongly than the other plants, though they all showed a tendency in that direction on the hottest days. Fig. 9 shows one of the more pronounced examples of this behaviour, for plant S on 29 June 1935.

Another feature shown more clearly by one of the plants, namely plant Q, is a rise in the extension rate at the very end of the night's growing period, giving the slightly increased slope shown in Fig. 10, which is cut short by the regular morning cessation of extension. This behaviour is closely associated with the rapid rise of temperature which occurs between the hours of 6 and 9 a.m. on clear sunny mornings. This feature can just be recognized in the records for the other plants on the few days on which it is most pronounced in the case of plant Q.

When the weather is unsettled and the sun overclouded, the

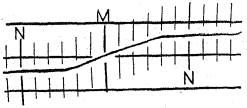

Fig. 7. Warm, clear weather.

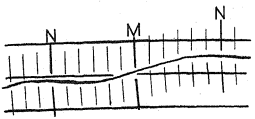

Fig. 8. Hot, dry day.

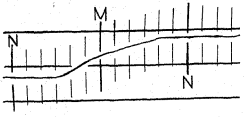

Fig. 9. Following a hot, dry day.

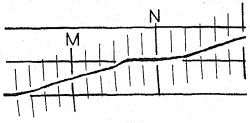

Fig. 10. Clear, warm morning.

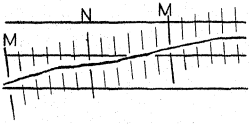

Fig. 11. Unsettled, overclouded.

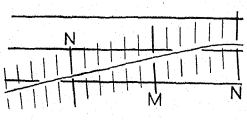

Fig. 12. No sunshine.

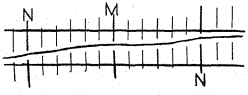

Fig. 13. Frost at night.

Figs. 7–13. Tracings of auxanometer records over periods of about 24 hr. *N*, noon; *M*, midnight. Fig. 7. Plant R, 28 June 1935. Fig. 8. Plant R, 10 August 1935. Fig. 9. Plant S, 29 and 30 June 1935. Fig. 10. Plant Q, 6 August 1935. Fig. 11. Plant S, 27 June 1935. Fig. 12. Plant P, 12 and 13 June 1936. Fig. 13. Plant S, 14 and 15 April 1936.

extension curves do not exhibit the regular characteristics just described; there is no sudden stop or start of extension, though there may be some diminution in rate as is illustrated in the curve for plant S on 27 June 1935 (Fig. 11). There have been short periods during the day when the rate was reduced to zero, and by experience of the behaviour of the plants, and by comparison with the temperature records, it is possible to attribute these occurrences to the temporary emergence of the sun. The curve for plant P on 12 June 1936, which is given in Fig. 12, illustrates a day during which the sun never shone, and the extension continues almost unchecked from one night to the next.

From what has been described it may be inferred that light intensity has a powerful control over the extension of the leaves during the daylight hours. On examining the records for the Mixed Sedge plants on fine days it appears that during June and July, extension ceases between 7.30 and 8.30 in the morning and begins between 5.30 and 6.30 in the evening (Summer time). This suggests strongly that extension is inhibited when direct sunshine falls on the leaves of the plant, but not when they are in diffuse light, for the plants grow close together in a dense vegetation into which the sunlight would not penetrate until the sun had risen to a considerable height. This idea is borne out by the fact that extension sets in earlier in the afternoon (4.0–5.0 p.m.) for plants P and Q than for R and S, the reason being, on this theory, that at this time they are shaded by the trees of the Carr which lies immediately to the south-west of their situation.

How far the magnitude of the extension rate is determined by other factors, when the light intensity is not so high as to inhibit extension, can best be judged by examining the variation in extension rate from day to day, or rather, from night to night. The data for plant S during the summer of 1935 have been worked out from this point of view and are presented in Fig. 14. At the top of the figure are shown the rainfall records from Fordham, and just below are the temperature readings from the Wicken thermograph; the maximum day and minimum night temperatures for the thermometer bulb in air are plotted at noon and midnight respectively on the appropriate dates, and between these lies the curve for temperature at 15 cm. depth, plotted directly from the original records. The next curve shows the total height increase that has occurred between noon one day and noon the next, the value for each 24-hr. period being plotted at the mid-point of the period. The values are the

actual heights in cm. obtained by the method of calibrating the record sheet described on p. 265. The next two curves show extension rates; the upper is the maximum rate attained during the initial phase of more rapid extension in the evening hours, but since this is not always exhibited the curve is not continuous. The lower of the two curves shows the steady value to which the extension rate falls, and maintains for the rest of the night. The lowest curve of all gives the "steady rate" values for plant P, included for comparison. These rates are obtained by measuring the angle of slope of the auxanometer record and multiplying its tangent by a factor which allows for the magnification at which the instrument is set, and for any error there may be in the rate of revolution of the drum. In order to show the degree of accuracy of this method, the measurement was made three times over for the dates from 3 to 8 July, for plant S, and all three alternatives plotted. It appears that all but the smallest variations in extension rate may be considered significant.

The curves for steady rate show a very marked correspondence with that for maximum day temperature. Not only does this hold in general over periods of several days, but a marked drop in day temperature is nearly always followed by a relatively lower steady rate value, as for example on 23 August. The day and night of 21 June illustrate the converse response to a sharp rise in temperature. There are one or two discrepant instances in the results for plant S, as for example on 11 July, where the rate curve is unaffected by a rise in day temperature, but the curve for plant P on the other hand does show the expected response. An explanation of this is not immediately apparent, but such cases are too exceptional to throw doubt on the main conclusion as to the correlation between day temperature and night extension rate.

The graph on the other hand shows no relation between the minimum night temperature and the extension rate, which is not surprising in view of the steadier temperatures prevailing underground where the growing region is situated. When the air temperature falls so low that the soil temperature is significantly lowered, it is probable that the growth rate is checked. Only one case of this sort is seen in the auxanometer records; it occurred in April 1936 when there was an exceptionally severe night frost. Fig. 13 shows the curve for plant S on this occasion. The records do not include the period during May 1935 when the growth of the plants was so markedly affected, but one must infer that growth during the night was checked at this time also.

Inspection of the curve for the total height increase during 24 hr. shows that there is a general parallelism between it and the rate curves. This means that on the whole the length of time during which the plant grows each night is fairly constant. There are well-marked exceptions to this, however, the most marked occurring on 1 and 5 July, and 8, 12, 22, 23, 26 and 30 August. In all these cases it is possible to explain the discrepancies in terms of weather conditions which have caused the extension curves to depart from the typical fine-weather form. Thus cloudy weather may prolong the growth period in the morning or cause it to begin earlier at night; sometimes the whole day is overcast and then extension continues all day. In cases where the height increase seems abnormally low as on 13 July, this is really due to the values on either side being abnormally heightened relative to the rate curve owing to slightly longer extension periods induced by weather conditions.

It is these values for total height increase which link up the results for actual extension rate at any instant with the weekly extension rates. In cool cloudy weather the proportion of the 24 hr. during which extension occurs is larger, but the lower temperature results in a rate which is so much lowered that it more than compensates for the extra time. It is for this reason that the weekly extension results show such a marked correlation with temperature (Figs. 1, 3 and 4).

The auxanometer results for the summer of 1936 are essentially similar to those for 1935, but show the typical fine weather curves much less frequently as the weather was usually unsettled. The records for periods of irregular weather, including those throughout the winter, can be interpreted without difficulty in terms of the weather record revealed by the temperature charts, on the basis of the conclusions which arise from the summer results already discussed. These are, firstly, that extension may be partially or totally inhibited during daylight hours, the degree of inhibition depending on the intensity of the light falling on the leaves; and, secondly, that its rate is roughly proportional to the maximum day temperature, above a certain limit, which, to judge from the data of Figs. 1 and 3, probably lies somewhere between 5 and 10° C. Thus it is very common in the winter months to find a week where a very slow extension rate has been steadily maintained, for the light intensity has never been high enough to produce a marked check during the daytime, though the temperature has not been so low as to prevent extension altogether.

Lastly, it remains to describe some results obtained from a plant growing in the shade of trees in the patch of woodland close to the plants P and Q. Records were taken from the middle of May 1936 till the end of July, in order to find out how the course of the daily growth rhythm is affected by conditions in the Carr. A thermograph was placed on the ground near the plant (plant T), for comparison with the temperature records from the Mixed Sedge.

Figs. 15 and 16 illustrate the results obtained. They give the data for two periods, one (29 May–2 June) the coolest, the other (17–21 June) the hottest, which occurred throughout the time of the investigation. Comparison is made between the extension curves for plant R in Mixed Sedge and plant T in Carr, the curves showing the height of the leaves in the same units for both. The two thermograph records are also shown. A subsequent comparison of the two instruments showed that the Carr thermograph gave a reading 4·4° higher than that of the other when they were placed side by side. It is clear of course from Figs. 15 and 16 that there must be some systematic difference of this kind, but it is also apparent that there are genuine temperature differences in addition. During the hotter period the Carr thermograph on two occasions gives a reading 5° or more above the other for the maximum day temperatures, while the difference between the night minima is less on the average, that is, about 3–3·5°. During the cooler week, on the other hand, there is never a difference of more than 4·5° C. between the day maxima, and the differences between the night minima range round 2·5°. That is to say, for given climatic conditions, the range of fluctuation of air temperature in the herb layer of the vegetation is larger in the Carr than in the Mixed Sedge. This may perhaps explain why the weekly extension curves for the Carr plants show larger fluctuations than do those for the other plants dealt with in Fig. 1.

If the extension curves for the hotter week are compared, it is clear that plant T has a much longer extending period each night, due to a much earlier start. The time at which extension stops in the morning is roughly the same for both, a fact for which only one explanation can be suggested, that is, that since plant T was near the north-eastern boundary of the Carr, the sunlight penetrated to it as early as it did to plant R, whereas in the afternoon plant T was shaded very much earlier.

The difference between the two plants in the evening hours is very much less conspicuous in Fig. 16, which gives results for a cool overclouded period. It is therefore possible to offer an explanation

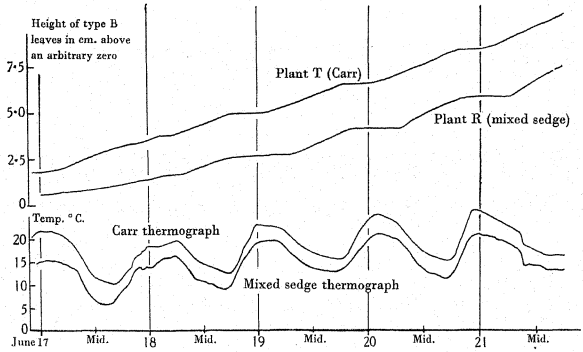

Fig. 15. Comparison between auxanometer records for a plant growing in the shade of bushes and another in the open sedge vegetation, with the records of shade temperatures in the two situations. 17 to 21 June 1936.

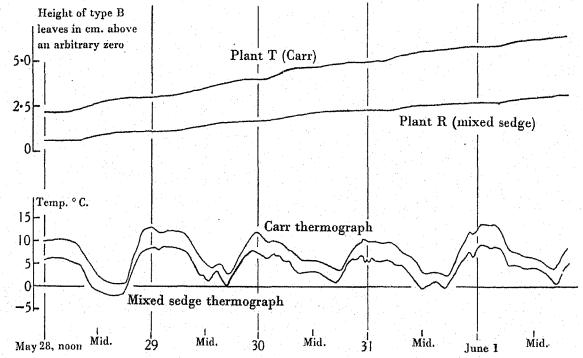

Fig. 16. Corresponding data to those of Fig. 15, for 27 May to 1 June 1936.

for the higher maximum rates shown by the Carr plants in Fig. 1. For it is during the periods of brightest sunshine and highest temperatures that the plants growing in the open will show the sharpest limitation of the nightly growing period, while the shaded plants will have a longer period, so that the effects of high day temperature in increasing the extension rate will be more apparent in the total extension for the week. The difference is enhanced also by the actual temperature differences already discussed.

Although, therefore, the lower light intensities in the Carr may be partly responsible for increased extension rates at times when the temperature is highest, this must be considered as an effect of etiolation, for the increased height of the leaves does not increase the general vigour of the plant. Thus plants G and H only produced four new leaves during 1935 in contrast to plant A which produced 5 and plant C 1 (fully watered) which produced 8. The low light is therefore considered to be the factor which in the course of time causes the *Cladium* plants in the Carr to become more unhealthy. This results in a lower general pitch of extension rate and for this reason a plant such as G, which has been in shade conditions for at least a year, only produces the same total increase in length of each leaf in one summer as plant A, although it has a higher maximum growth rate. This also explains the results for the plant in the greenhouse, shown in Fig. 4, for the conditions of diffuse light into which the plant was introduced in spring have lowered the power of its extension rate to respond to the higher temperatures which occur later.

DISCUSSION

The results which have been described show that the extending region of the growing leaf is exceedingly sensitive to external conditions. This sensitivity is shown not only by the way in which the extension rate is governed by the major seasonal drift of temperature and the lesser fluctuations of weather conditions from week to week, but also by the changes in rate which can be brought about in the course of a very few minutes by altering the conditions of illumination. Further, the differences between the local conditions affecting the plants in the different types of habitat in which it is found, are sufficient to cause recognizable distinctions between the extension-rate curves produced under these conditions. It is for this reason that the extension rate appears to be a very valuable indicator as to which habitats are most favourable to the species. This could of course in the long run be deduced by observing in which habitats

the species maintained itself in natural conditions, but in a place like Wicken where many of the plant communities are artificially maintained, it is useful to have some other guide. Ideally speaking, if one had the typical year's data for a plant which was growing in conditions where *Cladium* is known to be flourishing, then a comparison between this and the data for a plant in some other habitat should show immediately whether or not that habitat is genuinely favourable to the species. If it is not, then the occurrence of the species there must be fortuitous, that is, either it will soon become extinct, or else it is being maintained there artificially.

With regard to the actual ecological facts which have emerged from the data, we may notice first of all that the species appears to demand a completely waterlogged soil. Hence most of the area which is covered by *Cladium* at Wicken is sub-optimal for the species, because in a dry summer the water-table falls well below the soil surface. That the species is so abundant under these conditions is due to the removal of bushes by periodic crop-cutting. In the small area which can be classified as Pure Sedge, where the water-table is too close to the surface for colonization by bushes, the conditions for *Cladium* are better, but even here, a very dry summer such as that of 1935 lowers the water-table excessively. A lack of water is exhibited by a general lowering of the extension rates, though the latter always show the same type of response to temperature fluctuations.

There is one small place on the Fen which in regard to water supply is likely to favour *Cladium*, and this is the sloping shore and fringing banks of a small experimental pond. The latter was dug out just beside, and communicating with one of the dykes which bound the Fen. In these, the water is maintained at a high level throughout the summer and hence the roots of the *Cladium* plants growing there are probably growing in free water. It is therefore a rather striking fact that this is the one spot on the Fen in which *Cladium* flowers regularly and profusely, whereas inflorescences are very sparsely distributed over the *Cladium* communities in the rest of the Fen.

In the second place since the extension rate is so closely correlated with temperature it seems likely that, given ample soil water, the species could flourish still more in regions with a hotter summer than it does in our moderate climate, for the higher the extension rate, the more rapid the increase in assimilating area and therefore in general vigour of the plant.

This statement must be qualified by the conclusion which can be drawn from the data, that mere high temperature is not in itself the factor which favours the species, but the bright sunshine which is the cause behind high day temperatures. The plant is definitely intolerant of shade and this must be the reason for its gradual death under bushes and trees. Thus, in speaking of a high extension rate, it is necessary to distinguish between the occurrence at a given moment of an absolutely high rate—the mark of favourable conditions—and the appearance of a high rate when the average is taken over a period of some days—implying low light intensities and hence an inferior habitat or climate.

SUMMARY

1. Data are given concerning the rates of elongation of the actively growing (type B) leaves of *Cladium Mariscus* both over whole seasons and from day to day.

2. These rates vary greatly in magnitude and their fluctuations can be closely correlated with external conditions.

3. If the plant grows in soil which is not saturated with water it shows generally lowered extension rates compared with those of a plant growing in waterlogged soil.

4. The extension rate is closely correlated with temperature and hence shows a strong seasonal periodicity.

5. Extension is inhibited by strong light and therefore takes place mainly at night. The rate does not vary greatly in the course of any one night, and its magnitude is largely influenced by the temperatures of the preceding day. Hence the average extension rates over a week's interval fluctuate very markedly according to weather conditions.

6. Bright sunlight is a factor which favours the species, provided the soil water supply is sufficient.

Grateful acknowledgement is made to the Trustees of the Gordon Wigan Fund for a grant covering the cost of the auxanometers described in this paper.

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THE EMBRYO SAC OF *LIMNOCHARIS EMARGINATA* L.[1]

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(With 16 figures in the text)

Introduction

The development of the embryo sac of *Limnocharis* has already been investigated twice. The first publication is that of Hall (1902), who reported the development as of the *Lilium*-type (now designated as *Adoxa*-type[2]). His figs. 2–8 show correct draughtmanship, although his interpretation, as will be seen later, was faulty.

In a later paper Nitzschke (1914) reports that the embryo sac is formed from the third megaspore of a "T"-shaped tetrad and is six- or eight-nucleate at maturity. The former condition is due to a failure of the last division of the chalazal nuclei of the four-nucleate stage.

In a recent paper (Johri, 1936) I called attention to the apparent similarity between Hall's figures of *Limnocharis* and mine of *Butomopsis* and stressed the need for a reinvestigation of the former. In his review of the "types of embryo sac in angiosperms", Maheshwari (1937, p. 378) has accepted this idea and included *Limnocharis* under *bisporic* embryo sacs of the *Allium*-type.

Ovule. A longitudinal section of the mature ovule with the two integuments, nucellus and embryo sac is shown in Fig. 1. The integuments are free from each other as well as from the nucellus throughout their entire length. Nitzschke's (1914) fig. 16 shows a three-celled pro-embryo lying below the micropyle in direct contact with the cells of the inner integument and gives the impression that the nucellus disappears at this stage. This is incorrect because in my slides I have seen the nucellus even in embryo sacs with much older embryos. Narasimha Murthy (1933) made a similar mistake in *Limnophyton obtusifolium*.[3]

[1] *L. emarginata* L. = *L. flava* Buchen.
[2] See Maheshwari (1937).
[3] See postscript to my paper on *Limnophyton obtusifolium* (Johri, 1935).

Development of embryo sac. The hypodermal archesporial cell becomes recognizable at an early stage (Fig. 2). Sometimes two such cells may be found lying side by side or one upon the other. Nitzschke's fig. 17 shows a stage that could only have resulted from the presence of four archesporial cells.

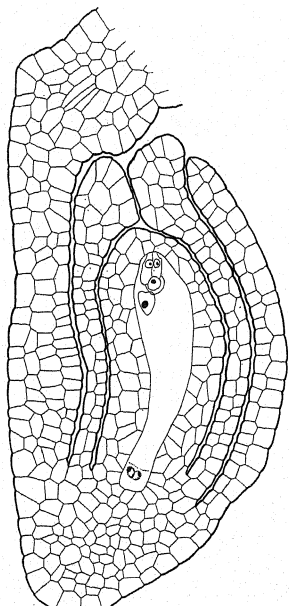


Fig. 1. Longitudinal section of ovule at mature embryo-sac stage. ×325.

The archesporial cell functions directly as the megaspore mother cell (Fig. 3). Its nucleus enters the prophase of the heterotypic division and a dyad of two cells is formed (Fig. 4). I did not find a wall cell in any of my preparations. Hall (1902) says: "An hypodermal

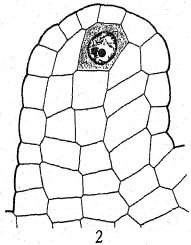
archesporium can soon be identified. A tapetal cell is cut off as found by Campbell in *Naias* and *Zannichellia*. In *Limnocharis*, however, the tapetal cell is without a wall, and it is pushed towards the apex of the sac, where it disappears in later stages of development." What Hall interpreted as the tapetal cell, seems to be the upper dyad cell which is of a small size from the very beginning and soon begins to degenerate (Fig. 5). The nucellar epidermis does undergo a periclinal division here and there, and when this occurs in a cell just overlying the megaspore mother cell, one may get a false impression of a tapetal cell (Fig. 7). Nitzschke (1914) also writes that a wall cell is cut off but gives no figures of the earlier stages. I am unable to confirm the observations of either Hall or Nitzschke on this point.

The nucleus of the upper dyad cell soon degenerates (Fig. 5). Occasionally it begins to divide but the degeneration advances at such a rapid rate as to intercept the process and not allow it to be completed. The nucleus of the lower cell undergoes further divisions to produce the mature embryo sac, which is thus of the "*Allium*-type" (older term, *Scilla*-type).

Hall (1902) writes that, "The large cell left after the formation of the tapetum becomes the embryo sac without further division", i.e. the embryo sac is of the *Adoxa*-type. Nitzschke (1914), on the other hand, claims that a T-shaped tetrad is formed but the two megaspores at the micropylar end and the one at the chalazal end degenerate and only the central functions. I have made a thorough study of the developmental stages but never saw a tetrad of megaspores.

During the division of the lower dyad cell (Fig. 6) a thin cell-plate is laid down on the spindle and the primary chalazal nucleus, which is always smaller than the micropylar, is pushed off towards the narrower end of the embryo sac, where it becomes cut off by a delicate plasma membrane (Fig. 7). Usually it does not divide further but promptly begins to degenerate and may be said to represent the single antipodal nucleus of the mature embryo sac (Fig. 14). Sometimes, however, it undergoes a mitotic division (Fig. 9) and gives rise to two nuclei in the chalazal cell (Figs. 11, 12, 15). Occasionally there seems to be an irregular mitosis or fragmentation of the primary chalazal nucleus (Fig. 13) increasing the number of nuclei in the lower end of the embryo sac to three or four (Fig. 16).

The primary micropylar nucleus divides twice (Figs. 8, 9) and out of the four nuclei produced, three organize into the egg-apparatus while the fourth enlarges and functions as the upper polar nucleus (Figs. 13, 14). The cell at the chalazal end contains one to three



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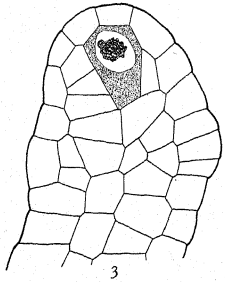

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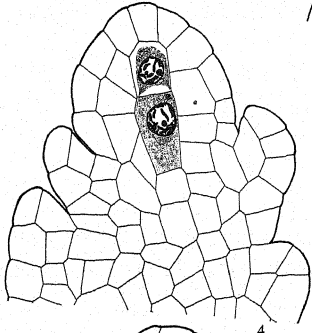

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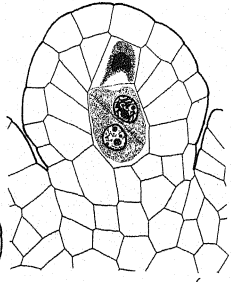

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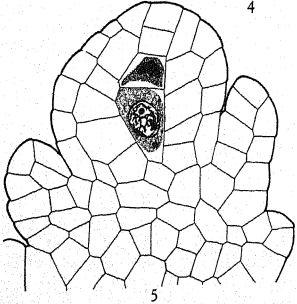

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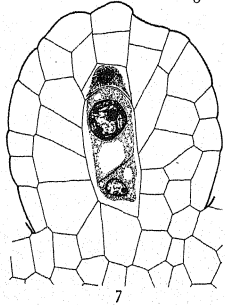

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(rarely four) nuclei formed in the method already described. Thus the embryo sacs are usually five-nucleate, occasionally six-nucleate and rarely seven- or eight-nucleate. I have never observed the upward movement of a nucleus from the antipodal end and believe that polar fusion does not occur normally.[1]

My observations on the later development of the embryo sac correspond very closely to those of Hall (1902), but his statement that "the antipodal is not cut off by a wall" is incorrect and is evidently due to oversight of the plasma membrane.

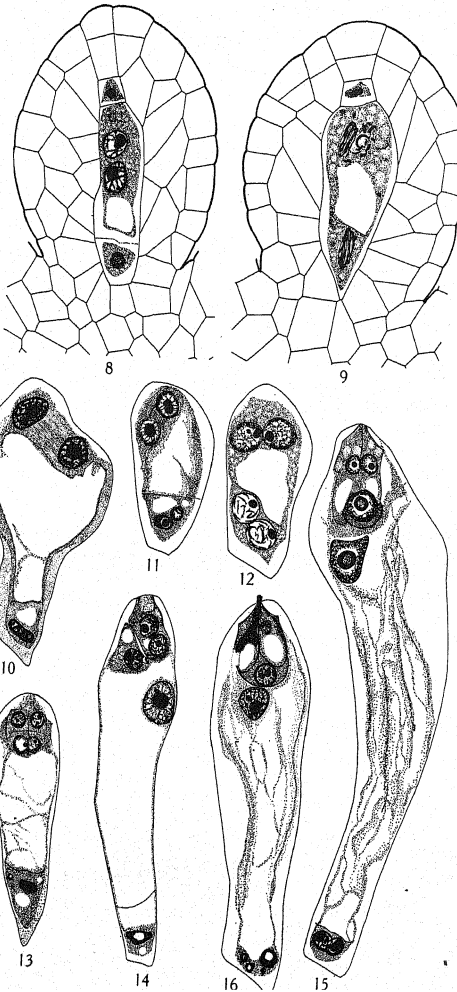
The observations recorded previously for *Butomopsis lanceolata* (Johri, 1936) and *Hydrocleis nymphoides* (1938), and in this paper for *Limnocharis*, show that the *Allium*-type of embryo sac is of general occurrence not only in the *Alismaceae* but also in the *Butomaceae*. The only exception is *Butomus* (Holmgren, 1913), which has been reported to have an eight-nucleate embryo sac formed from the lowest megaspore of a linear or a T-shaped tetrad.

Summary

A reinvestigation of the development of the embryo sac in *Limnocharis emarginata* shows that the previous accounts of Hall (1902) and Nitzschke (1914) are erroneous in some essential respects. According to the former author the embryo sac is *tetrasporic* and five-nucleate, while according to the latter, it is *monosporic* and usually six-, but sometimes seven- or eight-nucleate. My observations prove that *a tetrad of megaspores is not formed* at all *and the embryo sac is* BISPORIC, *arising from the lower dyad cell formed after the first (meiotic) division of the megaspore mother cell.* A true wall cell is not cut off, though Hall as well as Nitzschke have reported its presence. The nucleus of the lower dyad cell divides to produce the primary chalazal and the primary micropylar nuclei. As a rule, the former degenerates

Fig. 2. Longitudinal section of nucellus showing hypodermal archesporial cell. ×750.
Fig. 3. Longitudinal section of nucellus showing megaspore mother cell in synizesis. ×750.
Fig. 4. Megaspore mother cell divided into two dyad cells. ×750.
Fig. 5. Megaspore mother cell more advanced, showing degeneration of upper dyad cell; note that one of the epidermal cells of the nucellus has divided periclinally. ×750.
Fig. 6. Division of lower dyad cell. ×750.
Fig. 7. Two-nucleate stage; primary chalazal nucleus is smaller in size and is cut off at the base by means of a plasma membrane. ×750.

[1] Nitzschke (1914), on the contrary, shows two polar nuclei lying in the middle of the embryo sac in his fig. 15.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very early and is cut off by a plasma membrane from the rest of the embryo sac. The primary micropylar nucleus divides twice and produces the egg-apparatus and the upper polar nucleus. Thus, the mature embryo sac is five-nucleate, but occasionally the primary chalazal nucleus may divide or fragment, resulting in six-, seven- or even eight-nucleate embryo sacs.

I take pleasure in recording my grateful thanks to Prof. K. Suessenguth (Munich) and Miss Hester M. Rusk (Brooklyn Botanic Garden) for providing me the material on which these observations have been made; to my former teacher Mr Som Prakash, Professor of Biology at the Dayalbagh Intermediate College, Agra, for putting the entire resources of his laboratory at my disposal, and to Dr P. Maheshwari for suggestions and criticisms.

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Fig. 8. Three-nucleate stage, formed as a result of failure of division of the primary chalazal nucleus. ×750.

Fig. 9. The two micropylar nuclei as well as the primary chalazal nucleus in division. ×750.

Fig. 10. Same stage as in Fig. 8 but the embryo sac has grown exceptionally large. ×750.

Figs. 11–12. Four-nucleate embryo sacs; here both the primary micropylar as well as the primary chalazal nucleus have divided. ×750.

Fig. 13. Six-nucleate embryo sac showing beginning of organization of synergids. ×750.

Fig. 14. Five-nucleate embryo sac. ×750.

Fig. 15. Six-nucleate embryo sac. ×750.

Fig. 16. Fertilized embryo sac with eight nuclei; the four nuclei at the chalazal end (three small and one large) seem to have been produced from the primary chalazal nucleus by a process of irregular division. ×750.

A NOTE ON THE DEFINITION OF SUCTION PRESSURE

By A. B. BROWN, D.Sc.
University of Alberta, Canada

In recent botanical literature definitions of suction pressure (Sp) of plant cells fall into two groups, (*a*) those defining Sp in terms of a tendency for water to enter, and (*b*) those defining Sp in terms of actual entrance of water, when a cell with suction pressure is immersed in pure water.

Both types of definition are applied to exactly the same quantity (Sp), but manifestly they do not convey the same meaning.

A plant cell with suction pressure must of necessity absorb water if it is immersed in pure water or indeed any harmless non-penetrating solution with an osmotic pressure lower than the suction pressure of the cell. Definitions of suction pressure in terms of actual entrance of water, when the cell is transferred to pure water, are therefore correct.

Definitions in terms of a tendency for water to enter are undoubtedly misleading, and such definitions arise fundamentally from an attempt to distinguish between the tendency for water to enter the cell contents, and the tendency for water to enter the whole cell consisting of cell wall and cell contents considered as one unit.

The following definition of Sp is submitted as being unequivocal: The suction pressure of a plant cell is the force per unit area with which water would *begin* to enter the cell, if the cell were immersed in pure water.

REVIEWS

British Stem- and Leaf-Fungi (Coelomycetes). Vol. II. *Sphaeropsidales and Melanconiales.* By W. B. GROVE. $8\frac{1}{2} \times 5\frac{1}{2}$ in. Pp. ix+407, with 133 text-figures. Cambridge University Press. 1937. Price 21s. net.

With the publication of this second volume Mr Grove has completed the worthy but formidable task which he set himself, viz. to describe from the morphological standpoint all British fungi at present known that belong to the Coelomycetes. Mr Grove has recently had a serious illness and it is a great satisfaction to all mycologists that he has been able to complete so successfully the survey of this important group of the fungi. The treatment of genera and species in the present volume is essentially the same as in the first, but the references to recent literature are more comprehensive. The book will be a most valuable guide to all, mycologists and plant pathologists alike, who are concerned with these fungi.

As is usual with the author of this book, he expresses his opinions in no uncertain manner. The book is enlivened by the author's *obiter dicta* about the achievements of other workers and about modern methods of mycological and pathological investigation. Mr Grove's remarks on these and other topics culminate in an exhilarating but somewhat exasperating Epilogue. To state that "many of our plant pathologists have left the natural mode of work that was universal in earlier times, work out in the open" is a travesty of truth. Plant pathologists in general nowadays are no different from what they have been since real Plant Pathology began: from de Bary onwards they have known that pathogenic organisms must be studied both in the field and in the laboratory, and they have formulated their investigations accordingly.

Volume II terminates with a valuable index of the Ascomycetes with which there is proof or presumptive evidence that certain Coelomycetes are genetically connected.

F. T. BROOKS

A Textbook of Plant Virus Diseases. By KENNETH SMITH. Pp. x+615 and 101 illustrations. London: J. and A. Churchill, Ltd. Price 21s.

This work is intended to be read along with the author's *Recent Advances in the Study of Plant Viruses*, and the information in that work regarding methods of study and kindred subjects is not repeated here.

In a definite attempt to lessen the confusion in the study of plant viruses arising from a haphazard nomenclature the author propounds a scheme which, while not entirely new, is at any rate rational and comprehensive. Present knowledge is inadequate for any attempt at a classification based on the inherent properties of the viruses themselves, so an artificial system, based primarily on the first recognized (or most important) host plant and, secondly, on the reactions of the virus, is used. The system of nomenclature has been applied to all known plant viruses, and is capable of indefinite extension as new discoveries are made. For example the virus which causes Spotted Wilt of the tomato is called *Lycopersicum Virus* 3 Brittlebank. In this connexion it should be noted

that the author's name quoted refers to the first description of the disease, independently of the cause there suggested. In spite of the Latin form, the names, it is presumed, are in the English language, though this is nowhere specified. According to the preface the host plants are arranged in the text according to Hutchinson's classification, but no reference to this work can be traced in the many admirable and extensive bibliographies which are so useful a feature of the work.

The main properties of each virus are given and methods of transmission follow, with a list of the reactions of differential hosts where these are available. Description of the diseases caused in different hosts and lists of hosts are supplied in some detail. A commendable feature is the copiously illustrated chapter dealing with vectors, which should prove a great convenience to entomologists consulted on suspected insect carriers.

A general index is provided, together with an index of viruses and of authors. An appendix designed to make identification of virus diseases easier for the practical man is appended, but it has the drawback that it is only usable on the assumption that the practical man knows a virus disease when he sees it. The indexes have been tested but, with the exception of the index of authors, they appear to be inadequate, so inadequate as to interfere with the usefulness of the work. Every host plant, naturally or artificially infected, should be indexed if the real worth of the volume is to be realized. The old names of the viruses have not been included in the index of viruses, so that easy acquaintance with the new names is rendered more difficult.

The lists of hosts require a certain amount of botanical revision, though some of the errors appear to be due to uncritical carry-over from original work. It seems difficult to credit, for example, that there should be varieties of *Barbarea vulgaris* (p. 24) known as "Cress, True Water" or "Cress, Fine Curled", or that a form of *Aster laevis* (p. 280) should be "Aster, Ostrich Plume", when these names seem to suggest, unless Puck has been at our catalogues, *Nasturtium officinale, Lepidium sativum* and *Callistephus chinensis*. Perpetual Spinach is assumed on p. 41 to be a form of *Spinacia oleracea*, whereas it is the plant mentioned on the opposite page as Spinach Beet. The text suggests that the reference may be to the winter-hardy form of Spinach known as "Prickly". The cultivated strawberry is referred to as *Fragaria vesca* on p. 102, though the real nature of the plant is clearly stated on p. 104.

The work, on the whole, is commendably free from misprints, but on p. 104, line 16, Jardine should be Tardive, and Lefebre should be Madame Lefebvre. On the same page, 10th line from the bottom and on p. 111, line 13, *J. Agric. Sci.* in reference 19 on p. 398 should be *J. Agric. Res.* The omission of a semicolon on p. 280 makes the Broad Windsor Bean belong to the species *Dolichos Lablab* L. Witches' Broom is generally used with the first word in the plural, both in this country and in America. Here it is used in the singular throughout, which is pardonable; what is unpardonable is that many of the titles of papers quoted have been "edited" to the singular.

The appearance of this volume, the printing, and the illustrations are all to be commended, and the price is most reasonable. The work can be assured of a warm welcome from plant pathologists generally.

ALEX. SMITH.

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THE RESPIRATORY METABOLISM OF CARROT TISSUE. II.

THE EFFECT OF SODIUM MONO-IODOACETATE ON THE RESPIRATION AND FERMENTATION

BY JOHN S. TURNER
Botany School, Cambridge

(With 12 figures in the text)

I. INTRODUCTION

IODOACETATE was first used extensively in the experimental analysis of respiratory chemistry by Lundsgaard (1930). His valuable work on muscle tissue led him to experiment with yeast, and both he and Boysen-Jensen (1931), on the basis of their results with iodoacetate, abandoned the Pfeffer hypothesis of respiration. This hypothesis, elaborated by Kostychev and F. F. Blackman, has been widely accepted by plant physiologists, and it was obviously of importance to examine any fresh evidence bearing on its validity.

A full discussion of the problems raised by the experiments with iodoacetate, and a review of work done by the author and others, has already been published (Turner, 1937). The present paper gives some experimental results and recapitulates some of the arguments given in the review.

Slices of carrot-root tissue immersed in aqueous solutions of iodoacetate provided material. Details of the experimental methods, and of the preparation of the tissue, will be found in the first paper of this series (Turner, 1938).

Preliminary work has shown that the respiration of excised carrot slices probably does not differ qualitatively from that of normal uncut roots. The rate of respiration per unit weight of slice (suspended in aerated water) was high in freshly cut tissue and declined steadily with time. Here, however, the actual rates of respiration (R) and fermentation (F) are considered only in so far as they are altered by

iodoacetate. This is the procedure adopted in much of the work with animal tissue slices, and it seems probable that the plant slices resemble normal tissue much more closely than do the rapidly disorganizing tissues obtained from excised brain or liver, whose life after excision is relatively short.

II. THE INHIBITION OF FERMENTATION IN CARROT TISSUE CAUSED BY SODIUM MONO-IODOACETATE

When placed in water in equilibrium with an atmosphere of nitrogen, carrot slices produce carbon dioxide and alcohol by a fermentation process, which has been shown, by Wetzel (1933) and by El-Gawadi (1935), to be of the alcoholic type. The ratio of carbon dioxide to alcohol satisfies the equation:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH.$$

Aqueous solutions of iodoacetic acid, neutralized by sodium hydroxide, have the same effect on fermentation in carrot tissue and in yeast. They cause a progressive depression of the rate of fermentation, and in strong solutions fermentation is completely stopped. Experiments on carrot slices were made over a period of three years, the slices being cut from stored roots of varied origin. The tissue was washed in tap water for periods of from 4·5 to 600 hr. before the addition of the iodoacetate. In early experiments, in the Pettenkofer apparatus, the iodoacetate was added to the slices during a period of fermentation. In other experiments, and in all manometric experiments, the tissue was placed in nitrogen immediately after its contact with the iodoacetate solution. Media used included distilled water, acid phosphate and citrate buffers, and glucose solutions.

In all the manometric experiments the percentage inhibition of the fermentation rate was calculated from the data of two experimental curves, one obtained from untreated tissue, the other from tissue treated with iodoacetate. In most of the Pettenkofer experiments, no such controls were available, and the percentage inhibition of the fermentation rate was calculated by using as the normal value, the rate of fermentation just before the addition of the iodoacetate. This procedure is justifiable, because in these experiments the rate of fermentation had become fairly steady by the time the iodoacetate was added. Graphs showing the percentage inhibition of the rate of fermentation with time are given in Figs. 1–4, and they are discussed in the following sections.

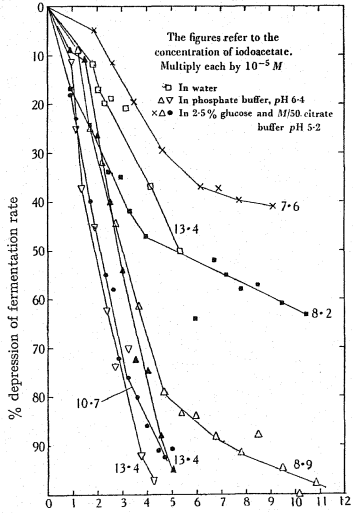

Fig. 1. The percentage inhibition of the fermentation rate plotted against time. Medium to low concentrations of iodoacetate. Manometric experiments. Temperature 22·5° C. Carrot tissue.

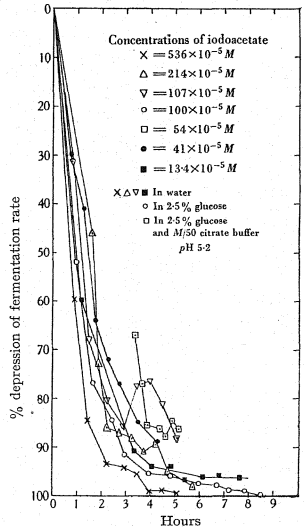

Fig. 2. The percentage inhibition of the fermentation rate plotted against time. High to medium concentrations of iodoacetate. Manometric experiments. Temperature 22·5° C. Carrot tissue.

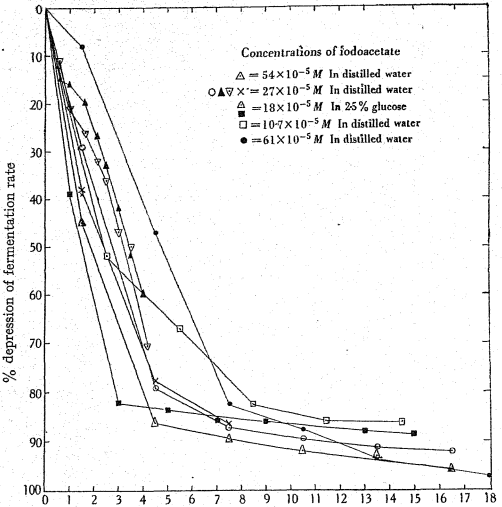

Fig. 3. The percentage inhibition of the fermentation rate, plotted against time. Medium to low concentrations of iodoacetate. Pettenkofer experiments. Temperature 22·5° C. Carrot tissue.

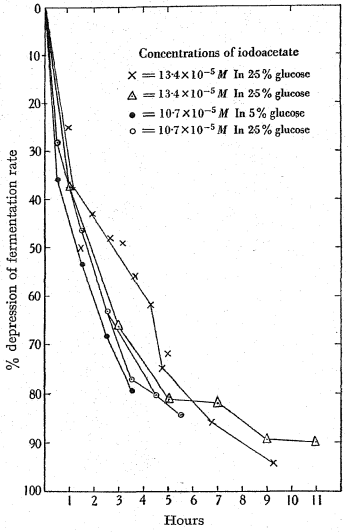

Fig. 4. The percentage inhibition of the fermentation rate plotted against time. Iodoacetate added previously to aerated tissue. For details see p. 18 and Tables IV, V. Temperature 22·5° C. Carrot tissue.

(a) "Residual" fermentation

After the addition of iodoacetate to carrot tissue in aqueous media, substances are liberated from the dying cells into the medium. There is in consequence always a possibility of the rapid multiplication of bacteria in the unsterilized medium. It is therefore desirable to make the period of anaerobiosis as short as possible, especially if the respiration rate following this period is to be measured. In most of the experiments the iodoacetate did not cause complete inhibition of the fermentation during the time allowed (Figs. 1–3). After 8–10 hr. the rate of fermentation was, however, very low, approximately only 5% of the normal rate. The "residual" fermentation still going on in the presence of the iodoacetate is not considered to be anything other than normal fermentation, capable of inhibition by iodoacetate. Its existence is believed to be due to the presence of cells in the centre of the carrot slices which are not reached by the iodoacetate at an effective concentration, even after 10 hr. We shall show later that there is evidence that iodoacetate does not penetrate yeast cells rapidly, even though these are exposed on all sides to the medium. The carrot slices were cut to a thickness of 1 mm. and were made up of about twenty-five layers of closely packed cells; the intercellular spaces were probably full of water and were mostly very small. It is known, moreover, that iodoacetate reacts in the cells with sulphydryl compounds and with amines, and it is probably adsorbed on enzyme surfaces; thus its passage into a tissue will be retarded by its destruction or fixation within each cell.[1]

In a few experiments the rate of carbon dioxide output rose rapidly, following an initial fall due to the application of the iodoacetate. In all these experiments, which were discarded, the medium became cloudy, and it was obvious that bacteria were present in large numbers. It may be argued that part of the residual fermentation in other experiments is due to the activity of bacteria in numbers not sufficient to make the medium cloudy. In the experiments recorded in this paper, however, such infection is extremely unlikely, for it was constantly observed, that once bacteria became active in the medium, their multiplication was exceedingly rapid and the

[1] As an example of the slow penetration of other soluble substances into carrot slices, we may briefly cite some experiments with methylene blue, which is also strongly absorbed by the cells. When carrot slices were placed in dilute methylene blue solutions, the innermost cells were unstained many hours after the outer layers had become a deep blue. This lack of staining in the interior was due, moreover, to the absence of the dye, not to its decolorization within the central cells.

medium became cloudy in an hour or two. In the experiments described here, the rate of fermentation fell continuously, and there was no cloudiness due to bacteria even after prolonged aeration of the poisoned tissues.

It is significant that concentrations of iodoacetate as great as those often used in experiments on yeast (100×10^{-5} *M*), caused complete inhibition of the carrot fermentation (Fig. 2). Moreover, in one prolonged experiment, 99% inhibition was obtained after 12 hr. in a concentration only $8{\cdot}9\times10^{-5}$ *M* (Fig. 1). The author feels fully justified therefore in assuming that the "residual" fermentation in shorter experiments with stronger iodoacetate solutions is that contributed by central cells not reached by iodoacetate, and that it is due neither to bacteria nor to the existence of any carbon dioxide-liberating mechanism unaffected by iodoacetate.

(*b*) *The concentration of iodoacetate*

If we assume that iodoacetate in very weak solutions is entirely absorbed by the cells, it is possible to compare the concentration (mols iodoacetate per g. tissue) required to inhibit fermentation in yeast and carrot tissue. Unfortunately, it seems that, for yeast, no thorough investigation of this point has been made. In most of the published work, the amount applied was 110×10^{-7} mol. per g. fresh weight yeast. Schroeder *et al.* (1933), however, reported that 22×10^{-7} mol. per g. fresh weight caused complete inhibition of fermentation after 2 hr. at a *p*H of 4·5, and from the nature of their enquiry it may be assumed that this was the lowest concentration which would cause complete inhibition. In the author's experiments, the corresponding quantity was $2{\cdot}7\times10^{-7}$ mol. per g. fresh weight of carrot, one-eighth of that required for yeast. The difference is not as great as might be expected, because weight for weight, yeast ferments much more rapidly than carrot tissue.

In this respect it is of interest to compare the amounts of glutathione present in the cells of yeast and carrot tissue. It is now well known that iodoacetate reacts with glutathione inside the cell, though from the work of Schroeder *et al.* (1933), it is doubtful whether this reaction is the cause of the inhibition of fermentation. As this question is not finally settled, it is important to note that the amount of glutathione in carrot tissue is so small that it is difficult and sometimes impossible to obtain a positive nitroprusside test with the juice extracted from the tissue even after its reduction with cyanide.

The figures are given in Table I, those for yeast from the paper by Schroeder *et al.*

TABLE I

Plant	Lowest concentration iodoacetate to inhibit F completely	Equivalent calculated GSH per g. fresh weight	Estimated GSH per g. fresh weight
Yeast	22×10^{-5} mol. per g. fresh weight	0·65 mg.	2·0 mg. Strong nitroprusside positive test
Carrot	$2{\cdot}7 \times 10^{-5}$ mol. per g. fresh weight	0·08 mg.	— Nitroprusside test very weak or negative

(c) The rate of inhibition of the fermentation

Carrot tissue in nitrogen has been treated with solutions of iodoacetate of concentrations ranging from 540×10^{-5} M to $6{\cdot}1 \times 10^{-5}$ M (equivalent to 140×10^{-7} mol. per g. carrot, to $2{\cdot}2 \times 10^{-7}$ mol. per g. carrot). These solutions all caused a progressive reduction in the rate of fermentation, tending rather slowly towards complete inhibition. The strongest concentration applied (540×10^{-5} M) caused the most rapid depression; the rate of fermentation fell to 1% of the normal rate in 2 hr., and to zero in 5 hr. A solution only 1/40 as strong as this, however, caused 80% inhibition in 2 hr., and 95% inhibition in 4 hr. The data for all the experiments are plotted in Graphs 1–3. As will be seen from the notes on these graphs, the conditions varied from experiment to experiment, for the experiments concerned were not planned to investigate simply the effect of the concentration of iodoacetate upon the rate of inhibition. Thus the *p*H, the composition of the medium, the length of the "Angärung" (the preliminary period of fermentation), and the age of the carrot slices were all variables. Table II summarizes the range of the effect produced after 2, 4 and 6 hr., for concentrations ranging from 9 to 540 times 10^{-5} M.

Lundsgaard (1932) has stated: "The attainment of an equal

TABLE II. *The percentage depression of the fermentation rate, in carrot tissue at 22·5° C., caused by solutions of iodoacetate of concentrations between 9 and 540 times* 10^{-5} M

That is, between 1 g. iodoacetate per 1000 c.c. medium, and 1 g. per 60,000 c.c. medium: Or between 140×10^{-8} mol. per g. and $2{\cdot}7 \times 10^{-8}$ mol. per g. carrot tissue (fresh weight).

Time after addition of iodoacetate, or change to anaerobiosis (hr.)	Percentage depression of the fermentation rate
2	13–89
4	33–97
6	55–100

degree of fermentation-inhibition in yeast, by the use of the same concentration of iodoacetate, at the same pH, on different research days is a matter of some difficulty." The same applies to carrot tissue, probably to a greater degree. Moreover, carrot roots, even when bought in bulk from one source (Turner, 1938), do not provide continuously comparable samples over long periods.

The variability in the effect of iodoacetate on the fermentation is also shown when concentrations weaker than 9×10^{-5} M are used, but these solutions do not cause so rapid or so complete an inhibition of the fermentation. Thus doses of 7 to 8 times 10^{-5} M caused between 40 and 60% depression after 10 hr. A concentration of $1{\cdot}3\times10^{-5}$ M had no effect on the rate of fermentation over a period of 10 hr.

All workers are agreed that under the most favourable conditions, a concentration of 100×10^{-5} M completely inhibits yeast fermentation within 1 hr. It has not so far been possible, using a wide range of conditions, to obtain such rapid inhibition of carrot fermentation. A concentration of 540×10^{-5} M caused complete inhibition in 5 hr., and more rapid inhibition was never obtained. It seems very probable that this is due to the relatively slow penetration of the iodoacetate into the carrot slices.

There is a good deal of evidence to show that even in yeast the rate of inhibition of fermentation is normally limited by the slow penetration of iodoacetate into the cells. Ehrenfest has offered convincing evidence to show that the increased rate of inhibition in acid buffers is due to the greater permeability of the cells under these conditions. Recently Goddard (1935) and Kohn (1935), have reported that the rate of penetration of iodoacetate into living cells is much lower than that of undissociated iodoacetamide.

It seems probable that the comparatively slow rate of fermentation inhibition in carrot tissue is also due partly to the use of tissue slices rather than cells suspensions. So far it has not been possible to increase the rate of entry of iodoacetate by placing the slices in acid buffers, or by increasing the period of Angärung. Both these treatments decrease the time necessary for the inhibition of yeast fermentation.

It may be argued that the iodoacetate enters the carrot cells as rapidly as the yeast cells, but that, owing to a buffering action, the carbon dioxide cannot leave the cells rapidly, so that an apparent slow inhibition of fermentation is recorded. It is important to state the evidence against this view:

(*a*) The rate of inhibition of the fermentation is a function of the

concentration of iodoacetate over the range employed. This is shown in Fig. 11 *F*, in which are plotted the results obtained in a series of experiments in which the conditions were uniform and the slices of similar origin. The time for 50% inhibition of the fermentation decreases as the concentration of iodoacetate increases. The effect is also seen in Fig. 5, where all the results obtained are plotted together as the percentage inhibition of *F*, after 2 hr., against the molar

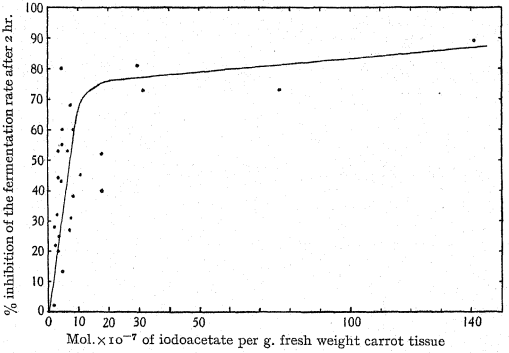

Fig. 5. The relation between the concentration of iodoacetate (mol. per g. carrot) and the percentage inhibition of the fermentation rate after 2 hr. Temperature 22·5° C. Carrot tissue. The curve is freehand.

concentration of iodoacetate. As stated above, the conditions in these experiments were very varied, and no analysis of the form of the relationship between inhibition and iodoacetate concentration has been made. The conclusion drawn from Figs. 4 and 5 is that they suggest that the measured rate of inhibition is not governed by the carbon dioxide-buffering power of the tissues, over the range of concentrations applied.

(*b*) The rate of loss of carbon dioxide from the tissues may be measured in experiments with normal material. In some experiments with normal carrot slices, in which the rate of carbon dioxide output was doubled or halved (following a change in the gas mixture supplied), the half-times for the establishment of the new steady rates of output were between 30 and 45 min. It is assumed that these

new rates are not established immediately because the tissue tends to retain the carbon dioxide. Hence if iodoacetate did penetrate the tissue instantaneously and cause inhibition of F, we should expect the measured half-time of the inhibition to be not more than 45 min. Fig. 1 shows that such a half-time was obtained only for the strongest concentration used; in most experiments the half-time was much longer than this. Thus for $8{\cdot}9\times10^{-5}$ M it was 3·2 hr. It is therefore believed that the delay in inhibition in the experiments quoted is associated either with a slow penetration of iodoacetate into the cells, or with its slow reaction inside the cells.

III. THE EFFECT OF IODOACETATE ON THE RESPIRATION OF CARROT TISSUE

In his second paper, Lundsgaard (1930*a*) showed that certain weak solutions of iodoacetate, which inhibited the fermentation of yeast, had little or no effect on the rate of oxygen uptake by the same material. This phenomenon will be termed, in what follows, the "differentiating" or "Lundsgaard effect." In the present research, the method adopted in the Pettenkofer experiments was to obtain two comparable samples of carrot tissue, and to place one under aerobic, the other under anaerobic conditions. When the rates of carbon dioxide output in both had become fairly steady, the same amount of iodoacetate was added to each vessel and its effects measured. It was then sometimes possible to transfer the poisoned respiring material from aerobic to anaerobic conditions and thus to observe the fermentation inhibition in this sample also.

It has already been mentioned that the addition of weak iodoacetate to fermenting carrot tissue may be followed by the rapid growth of bacteria, or sometimes of wild yeasts, the fermentation of which masks that of the carrot slices. Such foreign infection is even more troublesome when the iodoacetate is added to tissues in aerated water. Many of the early experiments were rendered worthless in this way. Bacterial growth is presumably initiated by the nutritive or growth promoting substances liberated from the carrot cells, and there is thus a suggestion that even weak iodoacetate has some effect on the aerobic carrot tissue.

Experiments in which bacterial infection occurred were discarded, but some seven preliminary experiments of the type described above gave results which suggested that a concentration of iodoacetate of $10{\cdot}8\times10^{-5}$ M (1 g. iodoacetate per 50,000 c.c. medium), lay in what

we shall term the differentiating region. Such a concentration had a slower depressant effect on the rate of respiration than upon that of fermentation. A convincing differentiation was, however, not obtained in these early experiments, in all of which the medium was distilled water containing iodoacetate.

It was then observed that the Lundsgaard effect had only been demonstrated for yeast in contact with sugar, and in the author's later experiments glucose was added to the suspension of carrot slices. In three experiments, the iodoacetate and the glucose were added at the same time. It might be thought that such a procedure would bring about infection by bacteria, but it was not so. Sterilized sugar solutions were used and the experiments were made as short as possible. The glucose had no appreciable effect in altering the rate of inhibition of the fermentation, but it appeared to protect the aerated tissue from the iodoacetate. Three Pettenkofer experiments done in this way were successful in demonstrating the existence of a differentiation in the effect of iodoacetate upon F and R. No bacterial infection occurred in these experiments.

Experiment P 20 was typical. In Fig. 6 are plotted the rate curves of R in two samples A, B, of carrot tissue in water. When glucose was added to both, the rate of respiration of both A and B rose, although iodoacetate was present with the glucose added to B. When both samples were placed in nitrogen the fermentation rate of B fell below that of A, which was in pure glucose solution. The differentiation is seen more clearly in Fig. 7, where the ratios of R and F in Exp. P 20 ($B/A \times 100$) are plotted. The iodoacetate caused a depression of the respiration rate, but a much more marked depression of the fermentation rate. A similar ratio curve, obtained from Exp. P 21, is shown in Fig. 8. In Exp. P 23 a very well-marked differentiation was obtained. The ratio curve (Fig. 9) shows that iodoacetate had no effect on the respiration, but later caused, in the same tissue, an almost complete inhibition of the fermentation.

Such complete differentiation was not obtained again, even after prolonged experiment. But the partial differentiation of Exp. P 20, P 21 is typical of many results.

At the conclusion of Exp. P 23 work with the manometric apparatus was begun, and further attempts to repeat the Lundsgaard effect were made. These confirmed the view that the effect of iodoacetate on respiration is very variable. A series of experiments was planned to make the conditions as uniform as possible. The medium chosen was 2·5 % glucose in $M/50$ citrate buffer of pH 5·2. An acid

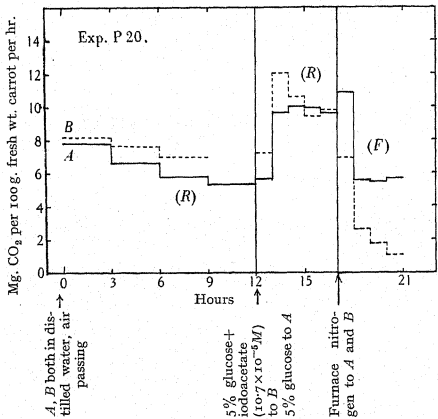

Fig. 6. The Lundsgaard effect. The rate of respiration (*R*) and fermentation (*F*) in the presence of glucose and iodoacetate, and with the rates in glucose only. Temperature 22·5° C. Carrot tissue.

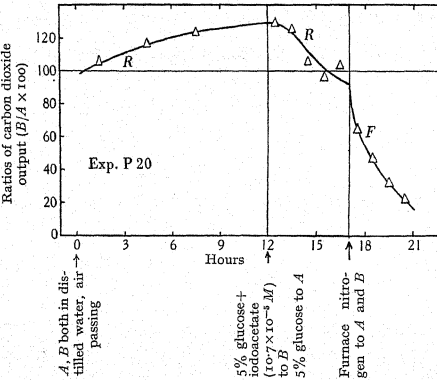

Fig. 7. The Lundsgaard effect. The ratios of the rates of carbon dioxide output by two comparable sets of carrot tissue, one treated with iodoacetate; calculated from the data of Exp. P 20, Fig. 6. Temperature 22·5° C. R: tissues respiring; F: tissues fermenting.

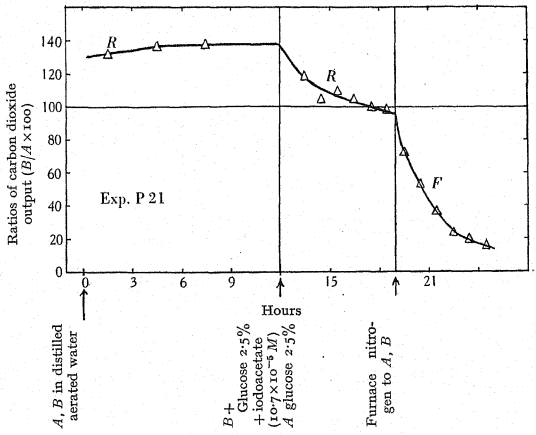

Fig. 8. The Lundsgaard effect. The ratios of the rates of carbon dioxide output by two comparable samples of carrot tissue, one treated with iodoacetate; calculated from the data of Exp. P 21. Temperature 22·5° C.

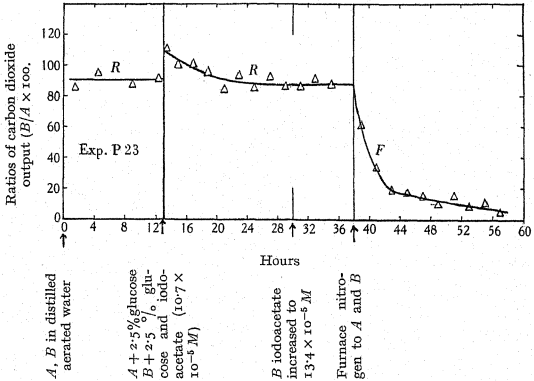

Fig. 9. The Lundsgaard effect. Complete differentiation between *F* and *R*. The ratios of the rates of carbon dioxide output by two comparable samples of carrot tissue, one treated with iodoacetate; calculated from the

buffer was used, as it is known that, in yeast, acidity increases the differentiating effect of iodoacetate. The slices were all cut at the same time from one set of carrot roots, and they differed only in the length of the initial period of washing. They were equilibrated with pure oxygen and 2·5% glucose solution for some hours before the experiments proper began. Respiration was measured as oxygen uptake in the manometric apparatus.

Each experiment was arranged as in Table III, below, and further details are given in Fig. 10.

TABLE III. *Plan of Exp.* M 26–M 29

A, *B*, in medium equilibrated with nitrogen		*C*, *D*, in medium equilibrated with oxygen	
A, glucose buffer	*B*, glucose buffer + iodoacetate	*C*, glucose buffer	*D*, glucose buffer + iodoacetate

Fig. 10 shows the ratios obtained from each of these four experiments. Continuous curves show the percentage depression of the fermentation rate with time, calculated in each experiment as the ratio of *B* to *A*. Dotted lines represent the percentage depression of the respiration rate with time, obtained from *C* and *D*. Four different strengths of iodoacetate were used.

The results are also plotted in another way in Fig. 11, where the half-times for inhibition (the time for 50% inhibition) are plotted against the concentration of iodoacetate. Line *F* gives the results for fermentation, line *R* for respiration.

These curves show clearly, that in this series of experiments iodoacetate inhibits both *F* and *R*, but for any given strength of iodoacetate solution the rate of inhibition of *F* is greater than that of *R*, For example, when *F* was completely inhibited by iodoacetate at $8{\cdot}9\times10^{-5}$ *M* (12 hr.), *R* was only 60% inhibited. This concentration of iodoacetate inhibited *F* at approximately the same rate as that at which $10{\cdot}7\times10^{-5}$ *M* inhibited *R*.

It was not possible, then, in this series to demonstrate complete differentiation, as in P 23. It might be possible to do so by using a suitable combination of *p*H, carrot tissue, glucose feeding and iodoacetate concentration, but it would be a matter of great difficulty. On the whole the evidence points to the following conclusion:

Iodoacetate has essentially the same inhibitory effect on F and R, but it acts more slowly on R than on F for any given external concentration. Certain experimental conditions increase the disparity, so that sometimes sharp differentiation, the Lundsgaard effect, is obtained.

Something is already known of the reasons for the variability of the Lundsgaard effect. It has been recently recognized that the

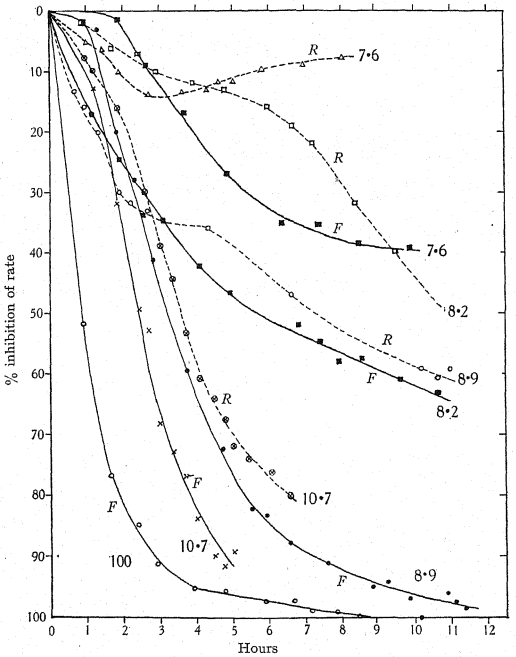

Fig. 10. The percentage inhibition of the fermentation rate (*F*, black lines) and respiration rate (*R*, dotted lines) plotted against time. Comparable samples of carrot tissue are used with each strength of iodoacetate solution. Manometric experiments. Temperature 22·5° C. Carrot tissue. The figures refer to the concentrations of iodoacetate applied, each $\times 10^{-5}$ *M*.

apparently complete differentiation obtained for yeast in early experiments, was due to the presence of alcohol. Lundsgaard himself showed that the yeast cell, in the presence of iodoacetate, can oxidize

alcohol. The uptake of oxygen accompanying this process was not formerly distinguished from that due to true respiration. Lundsgaard (1932) has now shown that the longer the time allowed for Angärung (i.e. the more alcohol present) before the iodoacetate is added to the yeast, the greater is the apparent differentiation.

Ehrenfest (1932) went further, and stated that any oxygen uptake occurring in the presence of the differentiating concentration of iodoacetate is due to the oxidation of intermediates previously

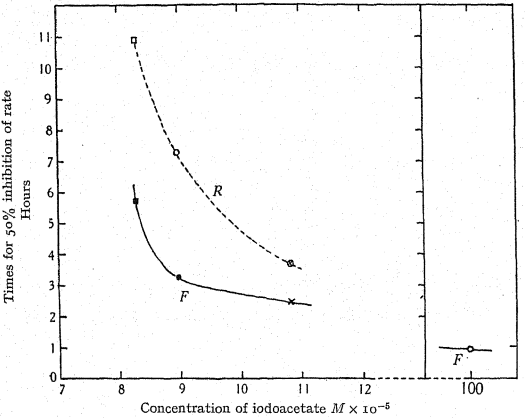

Fig. 11. Half-times for the inhibition of respiration (R) and fermentation (F), plotted against the concentration of iodoacetate. Calculated from the data of the experiments plotted in Fig. 10. Manometric experiments. Temperature 22·5° C.

accumulated in the cells or medium. But Lundsgaard believed that even when all the oxygen uptake due to alcohol oxidation had been taken into account, a differentiation in the effect of iodoacetate on F and R was still demonstrable. The author follows him in this respect, even though he has shown that added alcohol does raise the rate of oxygen uptake in carrot tissue treated with iodoacetate.

It can be shown that the R.Q. of carrot tissue in water is 1·02, and in glucose solutions equilibrated with oxygen it is raised only to 1·08. Hence in Exp. M 26 to M 29 (Fig. 10), differentiation

was obtained even when alcohol was present in only minute quantities. Nevertheless, it is interesting to note that the Lundsgaard effect becomes more marked as the amount of alcohol in contact with the carrot tissue increases. In the Pettenkofer experiments P 20–23 the tissues were in glucose solutions through which a current of air was passed. Under these conditions the R.Q. approaches 1·2, and it is in these experiments that a well-marked Lundsgaard effect has been demonstrated.

In explaining the differentiating effect of iodoacetate on fermentation and respiration, Lundsgaard treats the tissues as if they were enzyme mixtures *in vitro*; he assumes that an added external solution of iodoacetate at concentration X reaches the enzymes surfaces at a concentration X' whether the tissues are aerated or not. It is assumed that the iodoacetate inhibits triosis (glycolysis), and the supposed absence of effect on respiration is explained on the ground that triosis plays no part in respiration. The author takes a different view. Like Lundsgaard he assumes that iodoacetate acts on fermentation by inhibiting triosis, but he differs in accepting the Blackman Pfeffer hypothesis. He believes that when triosis is inhibited both respiration and fermentation are stopped. The difference in the effect of iodoacetate on F and R is then explained as being due to the effect of oxygen in modifying the "internal effective" concentration of iodoacetate. Thus a given concentration of iodoacetate is believed to have less effect on triosis when oxygen is present than when it is absent. Evidence of two kinds will now be presented in support of this interpretation. A discussion of the possible mechanism of the "oxygen effect" has already been published (Turner, 1937).

(*a*) *Pretreatment of aerated tissue with iodoacetate and the rate of subsequent inhibition of fermentation*

In some experiments it was possible to add iodoacetate at a concentration of $13{\cdot}4\times10^{-5}$ M (1 g. in 40,000 c.c. medium) to aerated cells without completely inhibiting respiration. Now according to Lundsgaard's hypothesis this would mean that, although the iodoacetate had inhibited the enzymes of triosis, the respiration, being independent of these enzymes, had proceeded in the normal way. If we allowed the drug to act thus for, say, 6 hr., then, on placing the same tissue in nitrogen without further addition of iodoacetate, there should be no further evolution of carbon dioxide in fermentation. Any carbon dioxide escaping from the tissues in nitrogen should be

merely that in equilibrium with the tissues at the moment of transference to anaerobiosis.

Reference to Tables IV and V, and the comparison of Fig. 4 with Figs. 1–3, will show, however, that the time lag in inhibition of F is not appreciably shortened by a pretreatment of aerated tissue with iodoacetate. Tables IV and V give details of four experiments in which the iodoacetate was added in air, when it caused only slight depression of the respiration rate. On transference to nitrogen, these tissues, which had been in contact with iodoacetate for from 5 to 25 hr., showed a rate of F almost as great, at first, as that of a

TABLE IV. *Details of the experiments in which the iodoacetate was added to aerated slices before their transference to nitrogen*

P 21. Iodoacetate, $10{\cdot}7 \times 10^{-5}$ M, added with 2·5% glucose and air passed for 7 hr. before nitrogen was given (Fig. 8).
P 20. Iodoacetate, $10{\cdot}7 \times 10^{-5}$ M, added with 5% glucose and air passed for 5 hr. before nitrogen was given (Figs. 6, 7).
P. 23. Iodoacetate, $10{\cdot}7 \times 10^{-5}$ M, added with 2·5% glucose and air passed for 17 hr. Concentration then increased to $13{\cdot}4 \times 10^{-5}$ M and air passed for another 8 hr. before nitrogen was given (Fig. 9).
M 19. Slices in contact with iodoacetate, $13{\cdot}4 \times 10^{-5}$ M in 2·5% glucose equilibrated with oxygen for 15 hr. Nitrogen then passed.

TABLE V. *Results of the experiments of Table IV, showing that the rate of inhibition of F is not materially affected if the drug is added previously under aerobic conditions. Controls taken from other experiments in which the same concentration of iodoacetate was used.*

Time after change to anaerobiosis, or after addition of iodoacetate hr.	Percentage depression of fermentation rate: Iodoacetate added previously in air, or oxygen (Table IV)	Mean %	Iodoacetate added in nitrogen	Mean %
2	43, 53, 60, 55	53	55, 25, 44, 13, 80, 32	42
4	75, 59, 79, 78	73	70, 96, 80, 33, 93, 60	72
6	83, 75, 86	81	96, 55, 70, 98, 98, 85	84

control sample without the drug. Table V shows, moreover, that the rate of fall of the fermentation rate in these experiments is little more rapid than it is in experiments in which the drug is added during anaerobiosis.

The experiments are unfortunately not quite comparable for they were carried out at different times, with different carrot tissue, and in

different media. However, it is not claimed that the pretreatment in oxygen has no effect whatever on the rate of fermentation inhibition, as is, in fact, suggested by the results set out, but merely that this pretreatment of the tissue does not cause the inhibition of the enzymes concerned in *F* before the tissue is placed in nitrogen.

It will be noted that experiments of this type tell against the view of Ehrenfest mentioned above. If the oxygen uptake in the presence of iodoacetate is all due to alcohol oxidation, the true respiration having been inhibited, then we should not expect the long lag in fermentation inhibition, following pretreatment. The existence of this lag led the author to the view that iodoacetate may penetrate the cells less rapidly in oxygen than in nitrogen; or that its action within the cell may be hindered under aerobic conditions.

(*b*) *The effect of low oxygen concentrations on the rate of inhibition of the fermentation by iodoacetate*

A much more direct and convincing demonstration of the effect of oxygen in decreasing the effectiveness of iodoacetate has been obtained in the following way. Carrot tissue shows almost as high a rate of fermentation in 2·5% oxygen as in pure nitrogen. It is therefore possible to investigate the inhibition of *F* by iodoacetate in the presence of a small amount of oxygen.

TABLE VI. *Manometric experiments*, M 30, M 31

Medium: phosphate buffer, *p*H 6·4. Temp. 22·5° C.

I. Normal tissue II. +iodoacetate, $13{\cdot}4 \times 10\ M$	CO_2 output measured in pure nitrogen
III. Normal tissue IV. +iodoacetate, $13{\cdot}4 \times 10\ M$	CO_2 output measured in 2·3% oxygen

V. Normal tissue: oxygen uptake measured in 2·3% oxygen

Two experiments were done, and in each five comparable sets of carrot slices were used. Table VI summarizes their treatment. No measurement of the rate of oxygen uptake by the tissue in 2·3% oxygen after treatment with iodoacetate was made, as the sixth vessel of the apparatus was required for the thermobarometer.

In Fig. 12 the results of both experiments are plotted together. The rate of *F* in poisoned tissue in pure nitrogen is expressed as a percentage of the rate in normal tissue in the same gas. Two curves are thus obtained, one from each experiment. The rate of "apparent *F*" in poisoned tissue in 2·3% oxygen is similarly expressed as a percentage of the rate of "apparent *F*" in normal tissue in this gas

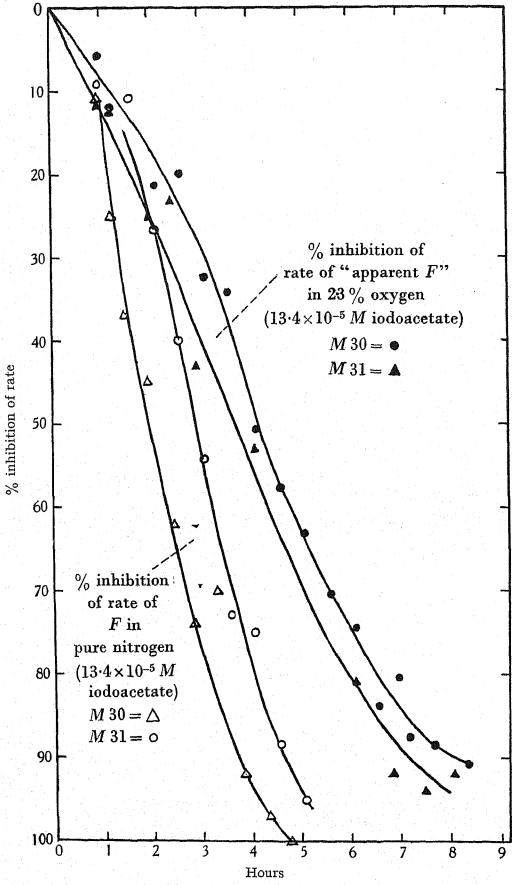

Fig. 12. The effect of oxygen on the rate of inhibition of the fermentation rate by iodoacetate. Manometric experiments. Temperature 22·5° C. Carrot tissue

mixture, and two more curves thus obtained. Comparison of the two pairs of curves shows the effect of oxygen in modifying the inhibition of fermentation by iodoacetate.

By "apparent F" we mean the actual increase of pressure in the closed vessel containing 2·3% oxygen, multiplied by the vessel constant. It is slightly smaller than the true fermentation in 2·3% oxygen, which may be calculated if we know the rate of oxygen uptake in the same vessel, the R.Q. of true respiration, and the solubilities of the gases concerned. At the beginning of the experiments concerned, the true fermentation in normal tissue in 2·3% oxygen was about 1·15 times the "apparent F", while at the end of the same experiment, when the oxygen concentration had fallen, it was about 1·05 times the apparent. It is unfortunate that the true fermentation rates in the poisoned samples in 2·3% oxygen could not be calculated as we made no measurement of their rate of oxygen uptake. For the purposes of the above comparison, therefore, we have used the ratios of the "apparent F" rates. This will only introduce an error of importance if the rate of oxygen uptake in 2·3% oxygen is increased in the presence of iodoacetate. All previous work shows that if the iodoacetate has any effect under these conditions it will decrease the rate of oxygen uptake. Hence the use of figures for "apparent F" tends, if anything, to bring the inhibition curves for nitrogen and oxygen closer together, and does not invalidate our conclusions.

Fig. 12 shows quite clearly that the presence of a small amount of oxygen caused a marked decrease in the rate of fermentation inhibition by iodoacetate. The rather large variability in the effect of the drug on the rate of fermentation, shown by the paired curves of M 30, M 31, is not sufficient to mask the oxygen effect. Three hours after the fermentation of the anaerobic tissue had stopped, the tissues in 2·3% oxygen continued to evolve carbon dioxide by fermentation at a considerable though falling rate. It seems very probable from the form of the curves that the oxygen, while lowering the rate of inhibition, does not decrease the actual extent of the inhibition.

The results of Exp. M 30, M 31 may also be expressed as follows: the half-times for fermentation inhibition in pure nitrogen were 2·1 and 2·9 hr., while in 2·3% oxygen they were 3·8 and 4·1 hr. The difference is of the order of that found for the rates of inhibition of F and R, caused by one concentration of iodoacetate.

The conclusion drawn from these experiments has already been stated in a previous paper (Turner, 1937). Iodoacetate is believed to cause inhibition of triosis, thus causing, on the Blackman-Pfeffer hypothesis, cessation of both fermentation and respiration. The fact

that respiration is not inhibited at the same rate as fermentation is believed to be due to the effect of oxygen in decreasing the effectiveness of the iodoacetate. In the presence of oxygen, even in small amounts, the living cell either becomes less permeable to iodoacetate, or the rate of reaction of the iodoacetate inside the cell is diminished. The view has been put forward that the reactive surfaces destroyed by iodoacetate are —SH radicles bound to proteins, and that in oxygen the concentration of these is lowered, but apart from the work of Rapkine (1933) there is little direct evidence as yet which bears on this subject.

SUMMARY

1. Dilute aqueous solutions of sodium mono-iodoacetate inhibit the fermentation of carrot tissue in nitrogen. The rate of inhibition and the concentrations required are compared with those for yeast.

2. Iodoacetate inhibits respiration in the same way as fermentation, if sufficient time is allowed for its action. Under some conditions a Lundsgaard effect may be obtained for carrot tissue; that is to say, a given concentration of iodoacetate has, in a given time, less effect upon the respiration than upon the fermentation. It is shown that this effect is not entirely explicable on the grounds that the poisoned sample is oxidizing alcohol.

3. Evidence is presented to show that the effectiveness of iodoacetate as an inhibitor of triosis (glycolysis) in living tissue is diminished in the presence of oxygen. Inhibition of the triosis may become complete in oxygen, but it takes longer than when the tissues are in nitrogen. The Lundsgaard effect is explained on the basis of this fact.

4. It is concluded that the results of work with iodoacetate do not in themselves necessitate the replacement of the Pfeffer-Blackman hypothesis by one in which respiration and fermentation are regarded as completely independent processes.

It is a pleasure to acknowledge the kindly help and encouragement given by Dr F. F. Blackman during the course of this investigation.

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STUDIES IN THE AUTECOLOGY OF *CLADIUM MARISCUS* R.BR.

V. THE DISTRIBUTION OF THE SPECIES

By VERONA M. CONWAY

Botanical Department, Westfield College, University of London

(With 2 figures in the text)

THE papers already published on the autecology of *Cladium Mariscus* have dealt with very diverse problems and with only a very few of the number which are involved in making clear the relationship between any particular species and the factors of its environment. It now seems desirable to conclude this series of papers by summarizing the results in relation to the known distribution of the species and its fossil history.

According to the literature, the species is found in Europe, Asia, North America, Africa and Australia and is commonly described as tropical and subtropical. Drude (1896) gives its common habitats as "Gräben, Teiche, seichte Flussuferstellen", and it is stated in the *Vegetation der Erde* to be a plant of the reed-swamps both in Africa (Cape Verde and Angola) and in several European regions such as Spain and the Carpathians. It seems thus in general to be associated with open water, although von Post (1925) states that it may occur in the marshy ground beside springs. The part that it may play in the succession from open water to dry land is illustrated in an account by Zobrist (1935) of a common type of hydrosere in Swiss lakes. *Cladium* enters into one of the earlier stages and is associated with *Phragmites, Carex elata, Schoenus nigricans, Lythrum salicaria, Filipendula Ulmaria* and other species which together make up a community almost precisely similar to that found in the corresponding habitats in the Norfolk Broads. Almquist (1913) has described the way in which the species occurs in certain Swedish lakes in Södermanland. He states that it favours the warmest spots and those that are

sheltered from the north wind, and forms almost pure stands except for scattered bushes of *Myrica*. The presence of young plants at some distance from the main groups suggests that the species is able to spread by seed in this locality.

Apart from scattered references to the species in ecological and floristic literature, an account has been given by von Post (1925) which summarizes the known facts of distribution in Europe and discusses their interpretation. Von Post makes use of the data to interpret the fossil history of the species which is described in the same paper.

According to the description, and the map of the distribution in Sweden, which are given in that paper, *Cladium* is strictly limited at the present day to regions with calcareous or at any rate basic soils. It is exceedingly abundant in Gothland, where it grows not only within the shallow-water regions of the lakes, but to some extent also in the slightly higher regions with wet, but not submerged soil. On the Swedish mainland, on the contrary, the species is generally less abundant, and is restricted to the deeper lying situations.

The work of collecting the records of the European distribution of the species has been done by von Post, and from them he has compiled the map which is reproduced in Fig. 1. This shows a marked abundance of localities round the Mediterranean coast, especially in the south of France, and along the valleys of the Rhône, the Danube, and the Po. It has also, on the other hand, a clearly "atlantic" component, and this map may be compared with that which Matthews gives to show the distribution of his "Oceanic-southern" element in the British flora (Matthews, 1937, Fig. 3). The scattered occurrences in continental Russia are also worthy of note.

The map shown in Fig. 2 is intended to amplify slightly the data for Great Britain which appear in von Post's map. It has been compiled from Druce's *Comital Flora of Britain*, Watson's *Topographical Botany*, Praeger's *Irish Topographical Botany*, the records of the *Botanical Exchange Club*, the *Journal of Botany* and numerous local floras. It cannot claim to completeness for several reasons. Many of the records are dated round about 1860, a few even earlier, and there is no information as to whether the species is still found in those situations. A few (about 5%) of the localities, could not be found on the map, and again, certain counties were stated in Druce's *Comital Flora* to have yielded *Cladium* records, but these records could not be found in the literature. For such counties or vice-counties a circle has been inserted on the map in the middle of the

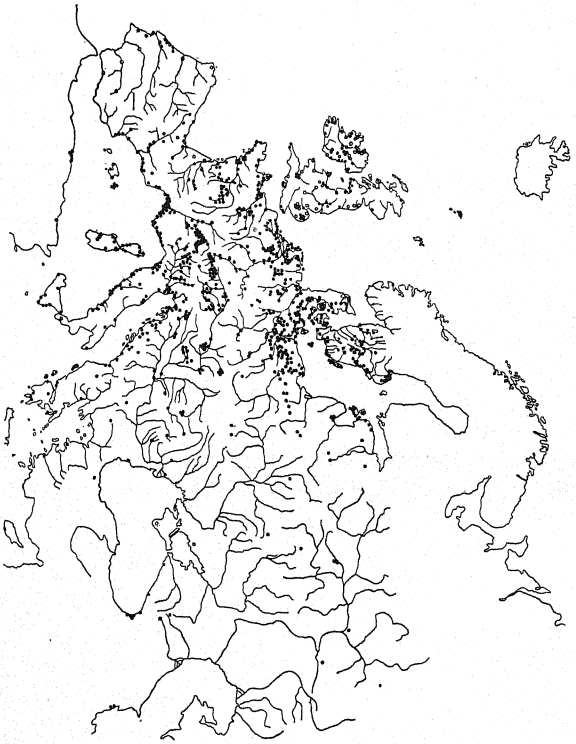

Fig. 1. Distribution of *Cladium Mariscus* in Europe, after von Post. ● *Cladium* localities. ○ Records of *Cladium* which could not be exactly located.

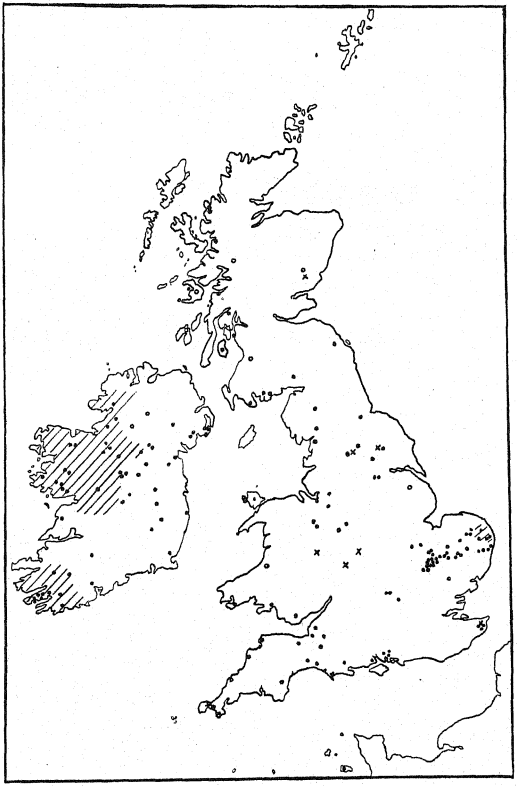

Fig. 2. Distribution of *Cladium Mariscus* in Britain. ● *Cladium* localities. ○ Records which could not be exactly located. × Localities from which the species is now absent, though formerly present. Hatching indicates those areas in which the species is stated to be frequent.

area. Sometimes the flora of a district states that *Cladium* is frequent in a certain area, and this has been indicated on the map by hatching. In a few instances it is definitely stated that the species has disappeared from a place where it was found formerly; such places are marked by a cross.

The map does however give an idea of the general distribution in Britain, and in particular it shows the regions in which *Cladium* occurs with relative abundance, namely the west coast and central plain of Ireland and the Fens and Broads of East Anglia.

Before attempting to analyse these facts in the light of recent work on the species it will be useful to summarize the views which von Post has put forward concerning its present distribution. To begin with, he considers that the abundance of the species round the Mediterranean indicates that it is favoured by warm summers. On the other hand, it is absent from large tracts of continental Europe where warm summers are the rule, and this he attributes to the more severe winters of the continental climate. He considers that low air temperature in itself is not harmful, but that the plant is damaged when the cold is sufficiently intense for frost to penetrate down to the depth of soil or water where the growing point of the stem is situated. He therefore thinks it important that in regions with winters as cold as those in Scandinavia for instance, the soil surface should be covered with a sufficient depth of water to protect it from frost; he suggests that this would explain why the species grows in areas just outside the lake margins in Gothland but not on the Swedish mainland, because in Gothland these areas are covered with water during the winter; in the rest of Sweden this is not so. Further, the rare localities in continental Russia may possibly be explained if they are characterized by exceptional conditions such as the presence of warm springs; certain instances of this type are in fact known.

Von Post quotes evidence to show that *Cladium* has died out over large areas because so much of the wet land in which it normally grows has been drained for cultivation. There are, on the other hand, places in Sweden where the species is reported to be spreading, so that it is not altogether clear whether it should be considered to be dying out, as one might think in view of the fossil evidence which will be discussed later.

Turning now to the experimental data which have been obtained, it is clear in the first place that the plant is truly aquatic, for it definitely flourishes more when its roots are below the level of the water-table than when they are merely in moist soil. This has been

demonstrated by the growth data described in Part IV of this series (Conway, 1937). Like other aquatics, the species shows a high proportion of internal air space relative to the total cell volume, and this is sufficient to allow oxygen to diffuse down to the submerged parts of the plant so that they are independent of the oxygen concentration of the external medium, which is generally very low in submerged soils.[1] The oxygen has, however, to diffuse downward through the leaves in order to reach any of the meristematic regions; further, until the time of flowering all the leaves spring from the submerged stock and are not borne up above the water on an elongated stem, so that both downward diffusion of oxygen and assimilation will be hindered in proportion to the depth to which the leaves are submerged. Although there is no evidence to prove it, it therefore seems very probable that the anatomy of the plant limits it to the rather shallower parts of the lakes in which it grows. This limitation is clearly seen if the situation of the species in the Norfolk Broads is compared with that of *Scirpus lacustris* and *Phragmites communis*.

If, then, waterlogged soil is a condition for the plant under natural conditions, the Mixed Sedge (Cladio-Molinietum) at Wicken Fen, must be suboptimal as a habitat for *Cladium*, and it is maintained there only because it is protected from the competition of the bushes which invade the community when not kept down by periodic cropping. The poor stature of the plants growing here confirms this idea by contrast with the tall growth which is shown in the *Cladium* associations of the Norfolk Broads.

In the second place, the evidence of quantitative observations all go to show that high summer temperatures are of benefit to the plant, thus confirming von Post's deduction from the distribution map. In fact, in view of the readiness with which the growth of the plant responds to increased temperatures, it seems as though climatic conditions in the British Isles are relatively poor. This applies particularly to Ireland where summers are for the most part cooler than in the more eastern parts of Britain. The map, Fig. 2, indicates a great abundance of the species in certain parts of Ireland, but this might possibly be fallacious, for when a local flora states that a plant is "abundant" it does not necessarily imply that it covers large areas as a dominant species. I have not been able to see the localities for *Cladium* in the Irish bogs, but Dr Godwin informs me that in the

[1] Evidence for this was obtained at Wicken, where the ground water never contained more than one-tenth of the value corresponding to equilibrium with the atmosphere and very often contained no oxygen at all.

extreme west it does not grow with anything like the luxuriance with which it flourishes in the Norfolk Broads. Be this as it may, it is undoubtedly of frequent occurrence in Ireland, and this may in part be due to the greater number of sufficiently wet habitats that are found there, in contrast to the small number in England, where drainage has probably been more intensive. Again, although the summers are oceanic, the mean annual temperature is as high over most of Ireland as it is in East Anglia, namely from 9 to 10° C., while for most of Scotland it is below 8°.[1] Possibly a longer growing season may compensate for the lower maximum temperatures, but it seems best to leave the question in doubt at present and not to place too much weight on this explanation of the Irish distribution, especially in view of the conclusion which has been reached in regard to light intensity as a habitat factor. The suggestion was put forward in Part IV, that bright sunlight is more favourable to the species than diffuse light, even though temperature conditions are the same. The conditions of high atmospheric humidity and consequent absorption of the incident radiant energy which characterize an extremely oceanic climate make an added difficulty in explaining why *Cladium* is abundant in Ireland.

The good effect of a sunny habitat does however explain why the species should succumb so rapidly when bushes invade its territory, even though the soil water conditions are adequate. Similarly, it becomes easier to understand why the species is rarely mentioned as a component of swamp vegetation in tropical and subtropical regions in spite of the fact that the general climatic conditions might be suitable. For here in contrast to the temperate regions there are tree species which can colonize water as deep as that in which *Cladium* flourishes, and so there remain few localities in which all the conditions for the species are fulfilled.

It seemed desirable to make an experimental test of the suggestion which was made by von Post concerning the sensitivity to frost of the growing-point, and through the kindness of Dr Kidd and Dr Barker, it was possible to carry out a number of experiments at the Low Temperature Research Station in Cambridge. The majority of the experiments were carried out in December 1934 and January 1935. The results of these are given in Table I, and the experimental details are as follows.

[1] Bartholomew's *Atlas of Meteorology*.

Experiment 1. *Plants* 5–8

Four shoots were transplanted from the Mixed Sedge at Wicken to a wooden box full of damp peat, 30 cm. square and 30 cm. deep. A glass tube just wide enough to hold a thermometer was pushed down between the plants so that the thermometer bulb should be at about the level of the growing points of the plants. A thermometer was inserted, and the projecting end of glass tube and thermometer were covered over by the cylindrical cardboard thermometer case. It was hoped that in this way the thermometer would give a reading of the soil temperature at the level of the bulb. For five nights the box of plants was left in the −10° C. chamber from 4.45 p.m. till 9·15 a.m. the next day. During the daylight hours it stood in a sheltered yard. This procedure was adopted because the freezing chambers are, of course, dark and it was not desired to complicate the results by leaving the plants for abnormally long periods in darkness.

The air and soil temperatures were read at 4.45 p.m. and 9.15 a.m. and during the week of this experiment the air temperatures ranged from 7·5 to 9° C. The soil temperature fell gradually until on the last day it was at −2° C. The plants were then left for 2 days in the yard, after which the soil temperature had risen to 2·5° C. The only symptoms observed were the folding together of a few leaves in their upper parts, and the appearance at the base of the younger leaves of a slightly paler green than is normal. The plants did not on the whole appear unhealthy. They were then subjected to 48 hr. at −10° C., after which time the soil temperature was −1·5° C., this did not produce any striking change in their appearance. They were brought back to the Botany School and kept in damp peat on the roof, and during the following summer they grew as healthily as many other transplanted *Cladium* plants which had not been exposed to frost experimentally.

Experiment 2. *Plants* 1–4

These plants were placed in a box of peat as in the preceding experiment, but this box was contained in a larger one 60 cm. square and 60 cm. deep, and the space between the two boxes was filled up with loose cork, so that the inner box was separated from the outer air by 15 cm. of cork in every direction. The whole was then put into −10° C. for five nights alongside the plants in Exp. 1. At the end of the 5 days the soil temperature was still as high as 2·5° C. The young leaves looked slightly pale at the base as did those in Exp. 1. Two plants (Nos. 3 and 4) were taken out of the box and No. 4 was kept in damp peat during the following months. It grew healthily during the summer. No. 3 was cut open to see whether any damage was visible, but all the tissues appeared perfectly healthy.

Two fresh plants (25 and 26) were added to Nos. 1 and 2, and the

cork covering replaced. After two days the whole was placed for one night at $-20°$ C. The soil temperature the following morning was $4·5°$ C. A few hours after they had been brought out of $-20°$ C. all the leaves had turned purple. One of the plants was removed and cut open and the growing point was found to have turned brown. Another was kept and the following summer normal growth was continued, although the parts of the leaves that had been exposed to the frost never recovered. The other two plants were unfortunately destroyed for another purpose.

Experiment 3. *Plants* 20–24

These plants, in a similar box of peat, were placed for a week in a well-lighted greenhouse on the roof of the Low Temperature Research Station. The temperature in the greenhouse was maintained constant at $-1°$ C. At the end of the week the soil thermometer registered $0·0°$ C., the soil being quite hard. The plants appeared healthy at the end of the week and four of them grew normally the following summer; the other one died.

Experiment 4. *Plants* 15–19

This experiment was precisely similar to Exp. 3, except that the greenhouse temperature was maintained at about $-4·5°$ C. By the fifth day the soil temperature had fallen to $-4°$ C. Although the plants did not look unhealthy when first taken out, they all died subsequently.

Experiment 5. *Plants* 33 *and* 35–38

Plant 33 was by itself in an ordinary plant pot and a thermometer was inserted in a tube close to the plant as in the preceding experiments. It was placed for two consecutive nights in the $-10°$ C. chamber, being left in the yard by day. By the end of the second night the soil temperature had fallen to $-7°$ C. This was much lower than the soil temperatures found in Exp. 1, a difference which must have been due to the much larger volume of peat contained in the wooden box than in the plant pot. The leaves of this plant showed the same symptoms as did those of plants 5–8, but it did not recover subsequently. The experiment was repeated with plants 35–38 each in a separate pot, with precisely the same result in every case.

Experiment 6. *Plants* 39–41

These three plants were placed together and their roots packed round with some damp peat and tied up in a piece of cloth. A glass tube with a thermometer was included as before. The basal parts inside the cloth were then embedded in loose cork contained in one of the foot-square boxes used in earlier experiments. The whole was then placed for two consecutive nights at $-10°$ C. together with

plants 35–38. The soil thermometer reading was only −0·5° C. when the plants were taken out of −10° C. on the second morning. These plants again had a few folded leaves when taken out, but they later appeared healthy and grew during the summer.

Experiment 7. Plant 42

It was thought that the destruction of leaves caused in Exp. 2 by −20° C. might be due to too rapid thawing after such a low temperature. Accordingly a single plant was placed at −20° C. from 9.30 a.m. till 4.30 p.m., then removed to −10° C. till 9.30 a.m. next day, and then left at −5° C. till 4.30 p.m. A few hours later, however, the leaves had turned purple as before, so that this idea could not be maintained.

Controls. Plants 10, 12 and 14

These plants were placed in a box of peat in the same way as in Exps. 1–4, but during the time of the experiments they were kept in a greenhouse adjacent to that used in Exps. 3 and 4, in which the air temperatures ranged from 5 to 12° C. The plants were watered just enough to keep the soil moist. They remained normal during this time and afterwards.

As may be seen from Table I, an attempt has been made to separate the effects of frost in the air and frost in the soil. It would have been better, ideally speaking, to design experiments which should fill up all the blank spaces in the table, particularly those for squares 2, 3, 8, 10 and 15; the others, which involve soils colder than the air above them, are less important since in natural conditions low soil temperature is obviously caused by lower temperature in the air above it. It would also be desirable to use much larger numbers of plants and to use intermediate temperatures, especially one between −10 and −20° C. The latter was not possible as no room was maintained at such a temperature. Also there were practical difficulties in the way of using larger numbers of a plant which is so large as *Cladium Mariscus*.

The results indicate first of all that provided the growing point is not subjected to temperatures below −2° C., an air temperature as low as −10° C. is not harmful. The one dead plant resulting from Exp. 3 does not seem sufficient to cast doubt on this general result. On the other hand, if the growing point suffers temperatures lower than −2° C. the whole shoot dies, even though the air temperature is not lower than −5° C. The experiments using −20° C. indicate that, provided the growing point is protected, the plant might make good the damage that is done so that the results might be considered

TABLE I

Soil temperature ° C.	Air temperature ° C.				
	Above +1	−1	−5	−10	−20
Above +1	(1) Controls Plants 10, 12, 14 All healthy	(2)	(3)	(4) Exp. 2 Plants 1–4 Nos. 2 and 4 healthy No. 3. probably healthy No. 1 ?	(5) Exp. 2 Plants 1, 2, 25, 26 No. 2 healthy No. 25 dead Rest ?
Between +1 and −2	(6)	(7) Exp. 3 Plants 20–24 4 healthy 1 dead	(8)	(9) Exps. 1 and 6 Plants 5–8, 39–41 All healthy	(10)
Between −2 and −7	(11)	(12)	(13) Exp. 4 Plants 15–19 All dead	(14) Exp. 5 Plants 33–38 All dead	(15)

inconclusive. Yet from the ecological point of view they are sufficiently conclusive, for if the plant grows in a place where temperatures of −20° C. occur at least once every winter, it will mean that all its leaves are destroyed once a year, and even though it produces new growth in the summer, it is most unlikely that the species could survive in natural vegetation under such conditions.

A few additional experiments were carried out during the first half of May 1935 in order to see whether the frost sensitivity of the plant is altered when it is growing actively.

Experiment 8

Plants 43–45 were in pots of damp peat and were left for 48 hr. at −5° C. No soil temperature was read but it is assumed that it fell nearly, if not quite, as low as −5° C. All the plants died.

Experiment 9

Plants 57–59 had their bases surrounded in damp peat and then embedded in cork as in Exp. 6 above. A thermometer was included also. They were given two periods of 24 hr. at −5° C., separated by 24 hr. in ordinary conditions. By doing this instead of giving them 48 hr. continuously, the soil temperature was prevented from falling below 0° C. All these plants remained healthy.

Experiments 10 *and* 11

These repeated Exps. 8 and 9 with plants 46–51, with the difference that the temperature −10° C. was used instead of −5° C. Also, owing to oversight, the plants in Exp. 10 were left for 72 hr. at −10° C. instead of 48 hr. These plants died, while those of Exp. 11 were healthy; the soil temperature in the latter case had not fallen below zero.

Experiment 12

Plants 53–56. These were again in plant pots and were put at −1° C. for 24 hr. They all died subsequently.

If these results are tabulated as shown in Table II it appears that, as in the winter, an air temperature of −10° C. is not in itself destructive, but at this season of the year the plant is not able to withstand even the least degree of frost which reaches its underground region, and it is to this extent more sensitive than during the winter period when it is not growing.

A comparison of these conclusions with the data on soil temperatures described in Part II (Conway, 1936*b*) shows that the *Cladium* will not be injured by the most severe winter conditions that are

TABLE II

Soil temperature ° C.	Air temperature ° C.		
	−1	−5	−10
Above 0		Exp. 9 Plants 57–59 All healthy	Exp. 11 Plants 49–51 All healthy
Between 0 and −1	Exp. 12 Plants 53–56 All dead		
Between −1 and −5		Exp. 8 Plants 43–45 All dead	
Probably below −5			Exp. 10 Plants 46–48 All dead

experienced at Wicken. The soil temperatures never fall below zero, and no temperature below −10° C. has been registered on the thermometer which is 5 cm. above ground, though possibly this thermometer may not be at the right level to register the lowest possible air temperature. It should not, however, give too inaccurate an idea of the temperature conditions for the herbaceous vegetation in which it is situated. As far as winter frosts are concerned, therefore, conditions at Wicken lie well within the limits tolerated by *Cladium*. Frosts during the growing period are the exception in this part of the country, and the experiments described lead one to expect that they might have an adverse effect on the plant. This expectation was strikingly borne out by the mid-May frosts which occurred in 1935. Later in the summer most of the leaves of the *Cladium* in the Fen showed a region, about 2 cm. long, which had turned brown, although the parts of the lamina above and below were normal in appearance. The injured region was at the same height on all the type *B* leaves.

By calculations made from plants whose growth-rates had been recorded, it could be shown that at the time of the frost this region of the leaves was just about at the soil surface, where the thermograph records show that the temperature fell to zero. The leaves must therefore have been sensitive in this region, which was presumably not meristematic, since the growing point is on the average 10 cm. below the soil surface. The cells in this region of the leaves would not yet have their walls thickened to their full extent, and

hence it can be understood that this part of the leaf might suffer more than the more distal regions.

Considering the limited nature of these experiments, the results seem surprisingly definite, and they confirm von Post's suggestion very strikingly. It is true that the general standard of frost resistance of the species might vary from place to place according to the way in which the plants are "hardened" by environmental conditions. But this would probably not alter the fact that the underground growing point is the most frost sensitive part of the plant, so that there seems little reason to doubt the correctness of von Post's idea.

A question which is not yet clearly analysed is that of the tolerance of the species to variation in soil reaction. The maps which von Post gives to show the occurrence of *Cladium* in Sweden, both now and in the past, indicate a strongly calcicole habit. The East Anglian localities are all within a region of neutral or alkaline ground water, and I am told by Dr Pearsall that the species is markedly basiphilous in its occurrence in the Lake District. All the evidence would seem therefore to point in one direction, were it not for certain exceptions. The chief of these is the statement by Lloyd Praeger (*The Botanist in Ireland*) that the species occurs in the "highly acid" waters of certain bogs in Connemara. Here again therefore the Irish occurrences seem divergent. Another case, possibly similar, is the description which is given by Skårman (1935) of the vegetation of the Bergsjö in Väster Gotland on the Swedish mainland. *Cladium* grows here with Phragmites in the reed-swamp zone, but on the shoreward side there are growing *Empetrum, Erica, Calluna, Vaccinium uliginosum, Eriophorum vaginatum, Sphagnum* spp. and *Rubus chamaemorus*. It is not stated that *Cladium* is growing in acid soil conditions, but it seems possible in view of the acidicolous character of the list just quoted.

As no other evidence is at hand concerning the *p*H range of the species, this difficulty has just to be stated and left unsolved for the time being.

Since it has been possible to confirm much of what von Post has provisionally suggested with regard to the present distribution, it becomes additionally interesting to consider the description he gives of the fossil history, and the way in which he interprets it. The data concerning the post glacial history of *Cladium* in Sweden are very abundant, not only because the species was widespread at certain times, but because a special search was made for the fossil remains during the intensive survey of the peat beds which was carried out by

von Post and his collaborators. The conclusions reached are therefore very well founded, and it is only regrettable that the same wealth of material is not available in other regions.

Cladium appeared first in Sweden at an early date; it came into the Swedish flora at the same time as *Corylus* and other species which followed closely on the willow-birch phase of the fini-glacial time. It rapidly became very abundant in all the calcareous areas which were above water, and remained equally abundant until the end of the Atlantic period, when it began to diminish very markedly. The evidence which shows the date of this dying out is derived mainly from the peat beds which can be related to the various stages of the land elevation following the Littorina transgression. It is found that in the areas raised up in the later stages, *Cladium* remains are absent, although there would seem to have been numerous localities which were suitable both in soil moisture and soil reaction.

This account applies to the mainland of Sweden, but in Gothland and Öland the *Cladium* remains have been found to be uniformly abundant throughout post-glacial time.

The history of the species on the mainland indicates that it was a characteristic of the post arctic warm period, which is often considered to have had a continental type of climate in Europe. Von Post, however, holds that since *Cladium* requires mild winters, judging by its present distribution, the boreal period in Sweden must have had a type of climate at least as oceanic as that prevailing in the more western parts of Europe to-day. Further, he ascribes the extensive dying out of the species to the onset of more continental conditions with the subboreal period. He considers the possibility that it might have been caused by drying out of the soil surface by peat accumulation, or by leaching out of chalk from the soil so as to produce more acid conditions. Both these he thinks might account for local disappearances, but the far-reaching character of the change in status of the species he thinks can only be ascribed to a climatic change. He supports this by the fact that *Trapa natans*, a species which to-day occupies the more continental parts of Europe, began to spread as *Cladium* was diminishing and reached a maximum in the subboreal period.

It is satisfactory that the results of the present investigations fall in so well with the work of von Post, and serve as added confirmation, if any is needed, of his use of the species as an indicator of climatic conditions in the past. It becomes of interest to know whether or not the plant is increasing its area at the present day, or whether it is doing so in one place and not in another. Unfortunately, this

problem is very much obscured owing to the removal of suitable habitats by drainage and other agricultural activities. There is however the evidence which Almquist brought forward in support of the view that in parts of Sweden the species is increasing its area. Moreover though such evidence is lacking at present for England, the plant does not give the impression that it is on the decline, if it is observed in the genuinely favourable localities which are found in the Norfolk Broads and elsewhere.

It is not clear either, of course, whether an increase in area implies an increase in summer temperature or an increased mildness of winter, though with regard to the British Isles it would be more reasonable to take the former interpretation. It is not often however that there are as few as two alternative explanations of the spread of a species, so that *Cladium Mariscus* may perhaps be considered as a species which is worth some attention from field botanists, and this not only on account of its use as an indicator of climate, but because of the biological interest attaching to its reactions to environmental conditions.

SUMMARY

The present distribution of *Cladium Mariscus* is discussed on the basis of the data published by von Post (1925). The main points in von Post's interpretation of this distribution are first that the species is benefited by high summer temperatures, that is, a mean temperature of 14–16° C. in the warmest months, and second that the growing point of the stem is frost sensitive so that the species is excluded from localities where frost is so intensive that it can penetrate to the underground regions of the plant. Experimental evidence is brought forward which confirms these views; in particular it is shown that the growing point is injured by temperatures below −2° C. though the differentiated parts of the leaves can withstand −10° C. This applies to the winter; during the growing period the growing point cannot stand any degree of frost. Hence, support is given to the idea that since *Cladium Mariscus* was very abundant in Sweden during the Boreal period, the latter must have been characterized, in Sweden at any rate, by a warm but oceanic type of climate.

This paper concludes a series dealing with the research done during the tenure of a Yarrow Research Studentship at Girton College, Cambridge. The problems investigated were suggested by Dr H. Godwin, and I am very greatly indebted to him for his continued interest and advice during the course of the work.

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NOTICE

"DATA FOR THE STUDY OF POST-GLACIAL HISTORY"

RESEARCH workers in the field of post-glacial history are concerned with piecing together evidence from many different sources, and making from it a consistent picture of development. It is only in the later stages of this process that the interpretation of results becomes obvious, and investigators in pollen analysis and bog stratigraphy often feel rightly diffident about premature explanations of their results. This, not infrequently, delays publication for a long time notwithstanding the fact that the publication of such data is an essential step towards a final co-ordinated scheme of post-glacial history. This is especially so when reasons of opportunity or convenience have led to the investigation of some isolated area, which it is not likely that the investigator can soon compare with nearby localities or with local geological or archaeological events.

The Editors of the *New Phytologist* therefore propose to encourage the production in this journal of a series of *short* notices under the title "Data for the Study of Post-glacial History". In any notice appearing under this title, it will not be expected that more need be given than the relevant facts of position, bog-stratigraphy, and the data produced by identification of plant remains or by pollen analyses, etc. It will not be considered necessary to suggest presumed ages for different parts of the deposits described, and indeed, this should be, on the whole, avoided. The written part of the paper should not, as a rule, exceed 1200 words, and might often be less. The first example, printed in this number, is regarded as of rather different kind from what we hope to make most of the series.

The Editors, in making this proposal, have in mind especially the accumulation of evidence for the post-glacial history of British vegetation, but they do not wish to exclude geological, climatic, archaeological or other similar evidence which must be produced in correlation with it.

If intending contributors to the series are in doubt as to the exact intention of the Editors, they can obtain further information by writing directly to us.

Whilst the exact method of presentation of the results of pollen analysis must depend on the nature of the site, the Editors think it

Limnische Sedimente.

Ton, feinkörnige minerog. Sed. im allgem.

Mergel.

Sand.

Kies.

Moräne.

Tongyttja.

Diatomeenerde.

Algengyttja.

Schneckengyttja.

Kalkgyttja.

Seekreide.

Feindetritusgyttja.

Gyttja im allgem.

Grobdetritusgyttja.

Schwemmtorf.

Feindetritusdy.

Dy im allgem.

Grobdetritusdy.

Sphagnumdy.

Braunmoosdy.

Telmatische Torfarten.

Equisetum-Torf.

Phragmites-Torf.

Magnocaricetum-Torf.

Cladium-Torf.

Sumpfdy.

Braunmoostorf.

Amblystegio-Caricetum-Torf.

Riedmoostorf, telmatisch.

Riedmoostorf, indifferent.

Terrestrische Torfarten.

Sphagnum-Torf, H 1-3.

Sphagnum-Torf, H 4-5.

Sphagnum-Torf, H 6-7.

Sphagnum-Torf, H 8-9.

Heidemoor-torf, H 10

S. cuspidatum-Torf.

S. imbricatum-Torf.

Ombrogener Hochmoor-Sphagnum-Torf.

S. fuscum-Torf.

Kiefernmoor-torf.

Bruchwaldtorf.

Erlenbruchwaldtorf.

Birkenbruchwaldtorf.

Terrestrischer Grasmoortorf, Parvocaricetum. usw.

Vaginatum-Torf.

Fig. 1.

would be an advantage if some degree of uniformity could be kept throughout the series. To this end they recommend that each tree pollen should be shown in a separate column, with the area between its curve and the vertical base line blacked in (*Corylus* excepted). If for any purpose it is desirable to give all the tree pollens on the same base line, the international symbols should be used. Depths should be given in centimetres, and, where practicable, it is suggested that the peat profile diagram should employ the symbols proposed by Faegri & Gams (1937), which are reproduced in Fig. 1. The English equivalents for the German terms are in most instances obvious, but the following notes may nevertheless be useful.

The terms "dy" and "gyttja", have had hitherto no satisfactory English equivalents. Both are organic muds formed in lakes or pools: distinction between them is based upon their origin, and it is sought to keep this evident by translating "dy" as *gel-mud*, and "gyttja" as *nekron-mud*.

Gel-mud is formed in oligotrophic waters by the precipitation of colloidal humic material entering the pool or lake in solution, very often as drainage-water from a bog-surface. Such oligotrophic waters are poor in plankton, and the gel-mud contains few remains of them.

Nekron-mud is formed in eutrophic waters by the accumulation of plankton-debris, or fragments from other components of the lake fauna and flora. The term "nekron" was first used in this sense by Sernander to convey the sense of a deposit composed of the bodies of plants and animals.

In eutrophic waters where the plankton is abundant, nekron-mud is formed most rapidly and the reaction of the water is alkaline enough to preclude gel-mud formation. Nevertheless intermediate conditions and transitional types of mud exist. "Grob-detritus dy" and "Fein-detritus dy" in the table of symbols, are characterized by a small to moderate nekron component in a deposit still essentially a gel-mud. The "Sphagnum dy" or Sphagnum gel-mud is the typical deposit from the bottom of pools in raised-mosses, and contains recognizable fragments of the typical *Sphagnum cuspidatum*. "Braunmoos dy" is a similar deposit with remains of Amblystegiaceae. "Sumpf dy" is not defined by Gams and Faegri, and, in the strict sense of our definition given above, can hardly be said to exist, as fen or marsh conditions preclude "dy" formation. What is here called "Sumpf dy" is probably well decomposed fen-peat.

The various types of nekron-mud are distinguished by the admixtures of other components with the plankton remains, or by the character of these remains: e.g., Ton gyttja = Clay nekron-mud, Diatom-erde = Diatom nekron-mud, Algen-gyttja = Algal nekron-mud, Schnecken-gyttja = Shell nekron-mud, Kalk-gyttja = Calcareous nekron-mud, Seekreide = Shell marl, Feindetritus gyttja = Fine detritus nekron-mud, Grobdetritus gyttja = Coarse detritus nekron-mud, Schwemm-torf = Drift peat. Other terms perhaps needing some comment are Mergel = Marl, Braun-moos = Amblystegiaceae, Heidemoor torf = *Calluna* peat, Bruchwaldtorf = Brushwood peat, or wood peat.

The use of the suggested symbols for different types of brushwood peat may be supplemented by the usual signs for erect or prostrate trunks where the evidence warrants it.

The editors are grateful to Professor H. Osvald for his advice with the terminology of the peat-types.

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THE SUBMERGED FOREST AT BORTH AND YNYSLAS, CARDIGANSHIRE

DATA FOR THE STUDY OF POST-GLACIAL HISTORY. No. 1

By H. GODWIN and L. NEWTON
From the notes of F. N. Campbell James

(With Plate III and 5 figures in the text)

(For some years Mrs Campbell James worked in the Department of Botany, University College, Aberystwyth, on the problems of the submerged forest of the neighbouring coast. She had intended to submit her results in a thesis for a research degree, but her untimely death prevented this. The authors consider the results of her work worth recording, even though they are incomplete, in the expectation that they will fit into a larger scheme based on wider investigation of the post-glacial history of British forests and of the British coast-line.)

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Introduction

The submerged forest at Borth and Ynyslas lies on the shores of Cardigan Bay just to the south of the Dovey Estuary. The area with which this investigation is concerned stretches intermittently for about three miles along the shore. The sea and wind take such very heavy toll of the forest that its extinction is but a matter of time.

Physiography of the area

The submerged forest lies about half-way between high- and low-tide marks on the sands bordering the "Dovey Flats". The Dovey Flats, as will be seen from the map (Fig. 1), consist of low tracts of land lying between the River Dovey to the north and the low foothills and headland of Borth to the south. Just south of the Dovey they consist of sandy marshes with a typical salt-marsh flora, and they merge gradually into the 2000 acres of Borth Bog (Cors Fochno). This latter is a raised bog (Hochmoor), with typical oxyphilous communities.

The river Leri crosses the margin of the bog on the seaward side. Entering the area to the south it runs in a northerly direction in an artificial channel until it joins the Dovey, keeping parallel to the sea for the latter part of its course. To the west of the Leri is a line of shingle beach, or storm beach, on which the village of Borth is built.

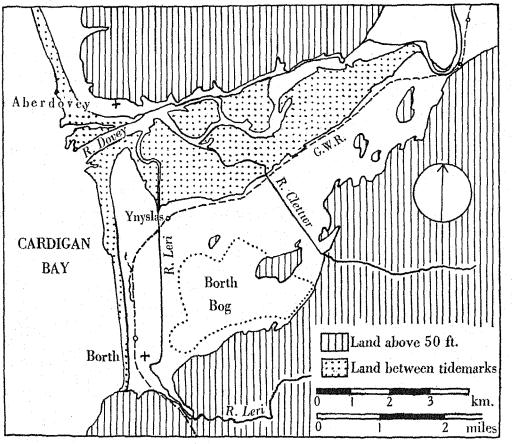

Fig. 1. Sketch-map of the area south of the Dovey Estuary, showing position of the submerged forest and of Borth Bog. Outcrops of the submerged forest occur between tide marks from Borth to Ynyslas.

To the north the beach is replaced by a line of sand dunes. There seems to be a constant drift of stones and sand toward the north (the prevailing wind is south-westerly) and this results in the formation of a bar across the mouth of the Dovey. Surrounding the whole area is a region of high ground. This consists of (1) the hills to the north, which rise steeply from the Dovey to the height of Cader Idris; (2) the foothills to the east, which run along the 50 ft. contour line and which mark the boundary of the post-Glacial deposits; (3) various "islands" of high ground on the edges and in the centre of the bog; (4) the headland to the south of Borth.

The surrounding islands already referred to, carry one of the roads round the bog, while one of the centre "islands" is known as Llwyn-y-garreg (the trees on the rock), and is extraordinarily interesting in that it shows how a rise of 5 ft. or so above the level of the bog enables an entirely different flora to flourish (see Yapp, 1911).

The submerged forest, which is frequently covered completely by sand, stretches intermittently along about two miles of coast. When fully exposed along its whole length (which seldom happens) it shows a flattened expanse of peat with many prostrate trees. To the south of Borth the peat abuts on rocks. The peat at the Borth end shows but few standing trunks, of which the majority are oaks.

It is probable that the hollows in the rocky base of the valley in which the bog is situated, are filled with boulder clay. Above this there appears to be a fairly continuous bed of "blue clay" or silt. This is found below all exposures of the forest which have been investigated, and it can be seen to run seaward. At very low tide it is seen to outcrop right on the sea edge. It is of unknown depth. To the south of Borth a boring 10 ft. below the peat of the surface, reached a hard mass and the borer brought up "boulder clay". One of the low-tide outcrops of clay at Ynyslas gave a clayey platform on which to bore and here, 8 ft. below the surface, the clay showed no change in composition and the borer refused to turn owing to the sticky nature of the clay. Deposits of an exactly similar nature occur below the bog whenever the depth of peat is not more than about 12–14 ft. The mechanical analysis of this clay or silt is shown below.

	Limits of diameter of particles (mm.)	Percentage
Fine gravel	3–1	Nil
Coarse sand	1–0·2	0·1
Fine sand	0·2–0·04	11·8
Silt	0·04–0·01	17·9
Fine silt	0·01–0·002	30·5
Clay	Below —0·002	20·6
Moisture	—	4·0
Loss on ignition	—	15·1

GENERAL STRATIGRAPHY OF THE SUBMERGED FOREST AREA

Both at Borth and at Ynyslas the general occurrence of the peat is similar. A layer of brown peat overlies the clay and merges gradually into it. The peat often consists of a mass of twigs and branches with or without bark, and is penetrated by roots; below, remains of *Phragmites* are abundant, and the upper surface is generally riddled with the holes of boring lamellibranchs. Trees are found abundantly with their stools and fallen trunks *in situ*, as is well shown in Plate III. *Pinus*,

Alnus, *Quercus* and *Betula* have been identified from the submerged forest at Borth, and four *Pinus* and three *Betula* (presumably seven separate trees) from the submerged forest at Ynyslas. The root systems of the larger trees are generally spread horizontally, though some also grow downwards. This is precisely the behaviour of trees growing in fen woods where the high-water table keeps all the tree roots (save alder) in the aerated surface layers of the peat.

The peat at Ynyslas is now usually 2 or 3 ft. thick; that at Borth is thicker.

(1) *The Ynyslas submerged forest sequence*

In November 1932 there was a good exposure of peat at Ynyslas,

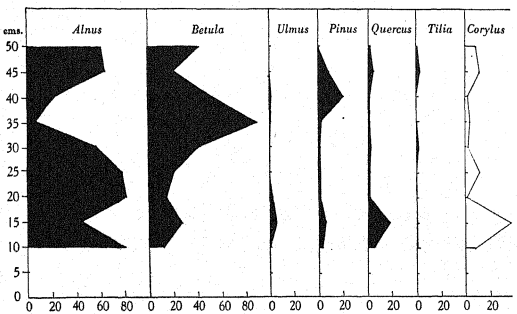

Fig. 2. Pollen analysis of profile in the submerged forest layer, Ynyslas. All pollen categories expressed as percentages of total tree pollen: this total excludes *Corylus*.

numerous patches being visible, generally in depressions filled with water. One fallen tree (*Pinus*) was lying *in situ* with its apex pointing eastwards and its roots intact.

Three borings showed the clay at 47, 44 and 51 cm.: a complete section was dug and examined at the third of these borings and samples were taken for the pollen analysis recorded below.

Each pollen sample (2·5 cm.) was treated with 20 c.c. of 5% KOH at 100° C. for 24 hr. The residue after straining through muslin was centrifuged and washed and then mounted in glycerine jelly. Three slides were made from each sample.

The results of analysis are given in Table I and Fig. 2. They are all expressed as percentages of the total tree pollen, but, owing to the

TABLE I. *Submerged Forest. Ynyslas.*

Depth (cm.)	Percentages of total tree pollen: *Alnus*	*Betula*	*Ulmus*	*Pinus*	*Quercus*	*Tilia*	*Corylus*	No. of pollen grains in total of 200: Tree total	M.	O.	M.+O.	Percentage of total pollen count of 200: % tree	% M.+O.	% M.	Comments and notes on forms not included in total of 200
50	56	42	—	—	2	—	8	72	40	88[a]	128	36	64	20	[a] 57 *Sphagnum* spores, 7 ericoid, 16 unknown
45	63	21	—	8	5	3	11	38	13	149[b]	162	19	81	7	[b] 66 *Sphagnum* spores, 55 ericoid, 24 unknown
40	23	53	1	21[c]	2	—	2	169	7	24	31	85	16	4	[c] *Pinus* maximum
35	7	90[d]	1	2	1	—	3	182	6	12	18	91	9	3	[d] Betula maximum
30	56	41	—	1	2	1	2	169	22	9	31	85	16	11	—
25	78[e]	21	—	1	1	—	11	152	22	26	48	76	24	11	[e] *Alnus* maximum
20	82[f]	15	5	1	1	—	1	151	34	15	49	76	25	17	[f] *Alnus* maximum
15	45	27	1	5	18	—	36	22	125[g]	53	178	11	89	63	[g] Monocot maximum
10	80[h]	12	—	2	5	—	9	127	42	31	73	64	37	21	[h] *Alnus* maximum
5	Grains too few to count. *Alnus* most prevalent, then *Corylus*. Occasional *Pinus*, *Betula*, *Quercus*, with few fern spores														
0	Clay base, horizontal rhizomes and erect axes of *Phragmites*. Little pollen but *Pinus* and *Corylus* could be distinguished														

M. = monocotyledonous pollen; O. = other pollen.

fact that 200 pollen grains *of all kinds* was made the total count for each sample, the samples vary much in respect of the number of tree pollens they include. It is at once apparent that the diagram shows broadly the lower half of the peat bed to have a dominance of alder pollen (10–30 cm.), followed by a phase of birch dominance (35 cm.),

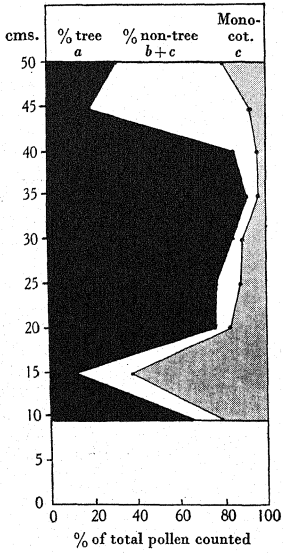

Fig. 3. Diagram of the Ynyslas profile shown in Fig. 2, expressing the relative proportions of tree pollen and of non-tree pollen of other categories.

then a small pine maximum (40 cm.), and lastly a return to high values for alder and birch. The basal clay contains foraminifera and is penetrated by *Phragmites* and the lower samples (especially at 15 cm.) show a very low ratio of tree pollen to non-tree pollen of monocotyledonous type. The peat from 20 to 40 cm., shows a very high ratio of tree pollen to other pollen (Fig. 3) which supports the idea that this was the horizon of the forest itself. The peat above 40 cm. shows

again a low tree pollen/non-tree pollen ratio, but the non-tree pollen here consists chiefly of *Sphagnum* spores or the pollen tetrads of Ericaceae. Microscopic remains of *Sphagnum* leaves are abundant and, together with *Calluna* twigs, show that acidic peat formed *in situ* over the forest peat.

The developmental sequence was apparently this:

(1) The formation of fen over the brackish-water basal clay, and the development of alkaline peat including *Phragmites*.

(2) The development of fen alder-woods on this peat—no doubt at first in patches and with a markedly wet reversion at 15 cm. In this reversion phase the tree pollen, wind-blown from the uplands, would play a greater part than in the succeeding phase of dense fenwood, and this would account for the relatively high values of oak, hazel and elm pollen. The monocotyledonous pollen from the fen itself quite swamps the total tree pollen under these conditions.

(3) Growth of the fenwoods *in situ*; at first alder, then birch and finally pine. This is in accord with the recognition of remains of these trees on the beach to-day.

(4) Development of *Sphagnum* peat (probably "raised bog") above the forest peat. Large raised bogs occur in the region at the present time (Tregaron Bog and Borth Bog).

It is to be noticed that the sequence of forest horizons, alder, birch, pine, and the corresponding tree-pollen maxima are well recognized on the Continent as a series occurring regularly at the transition from fenwoods to raised bog. It is well shown in the north-west German marshes (Brinkmann, 1934) and is recognizable also in the East-Anglian fens, where the subfossil pinewoods have been interpreted as having this status (Godwin *et al.* 1935).

In the view of the authors the pollen analyses do not yet permit of dating the forest except that it is clearly post-Boreal. Further knowledge of forest and bog development in Wales may however make these data of value. It may be noted in passing that Woodhead & Hodgson (1935) in the analysis of Snowdonian peats did not exclude the possibility of a post-Boreal pine maximum, and that Erdtman (1928) and Jessen (1934) have both recognized a secondary (sub-boreal) pine maximum in sites in different parts of the British Isles. It remains of course, for the present, an open question how far such pine maxima as occur are merely developmental phases of vegetation referable to any period, and how far they reflect by their prevalence, some specific climatic swing acting at the same time over a very large area.

There is no evidence in the pollen sequence of the submergence which brought the forest to its present position.

(2) *The Borth bog sequence*

The closeness of the present bog at Borth to the submerged forest suggested comparison of the pollen sequence in both, and in August 1933 a boring was made in Borth Bog through 4 metres of peat to the underlying clay. The samples for pollen analyses were prepared as follows: 0·1 c.c. of the sample was heated with 5 c.c. of 5% KOH for 3 or 4 hr. at 100° C., the test-tube was filled with water, and the sample left to stand overnight. Next day the settled residue was

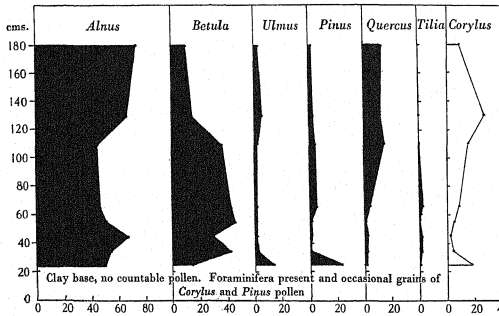

Fig. 4. Pollen analysis of profile in Borth Bog. All the pollen categories expressed as percentages of total tree pollen: this total excludes *Corylus*. The profile does not extend to the present bog-surface: the top 220 cm. were too poor in pollen to count.

shaken up with fresh water, and again left to stand. By a pipette the fine upper material was removed from the solid matter in the bottom of the test-tube;[1] this was spread on a slide and the water partly driven off by heating the slide on an asbestos plate. One or two drops of safranin jelly were mixed with the residue and a coverslip was added.

The results of analyses are shown in Table II and in Fig. 4. It should be noticed that only the lower half of the bog section is

[1] This method of preparation may involve errors due to the differential settling of the pollen.

TABLE II. *Borth Bog section*

Depth (cm.)	Percentages of total tree pollen							No. of pollen grains in total of 200				Percentage of total pollen count of 200			Comments and notes on forms not included in total of 200
	Alnus	*Betula*	*Ulmus*	*Pinus*	*Quercus*	*Tilia*	*Corylus*	Tree total	M.	O.	M. + O.	% tree	% M. + O.	% M.	
400–180	Tree pollen diminishes. Replaced by ferns, *Sphagnum* and moss spores, ericaceous pollen and monocots. *Myrica* leaves in upper layers.														
180	74[b]	10	3	—	13	—	10	31	38	131[a]	169	16	85	19	[a] 24 *Myrica*, 65 unknown. [b] *Alnus* maximum
160	Only tree pollen was found with a few ericaceous tetrads. Too little to count. [Omitted from graph]														
130	66	15	5	2	12	—	27	41	6	153[c]	159	20	80	3	[c] 65 *Sphagnum* spores. 53 *Myrica*
110	44	35	2	4	15	—	14	48	19	133[d]	152	24	76	10	[d] 107 *Sphagnum* spores
65	46	42	1	5	4	2	8	114	55	31	86	57	43	28	—
55	51	45[e]	1	1	1	—	5	142	36	22	58	71	29	18	[e] *Betula* maximum
45	67[f]	28	1	1	2	1	2	140	46	14	60	70	30	23	[f] *Alnus* maximum
35	54	41	2	—	2	1	4	129	47	24	71	64	36	24	—
25	50	14	14	23	—	—	18	22	157[g]	21	178	11	89	79	[g] Monocot, maximum. Mostly *Molinia* type
0– 25	Clay base, no countable pollen. Foraminifera present and occasional grains of *Corylus* and *Pinus*														

M. = monocotyledonous pollen; O. = other pollen.

represented, the upper samples containing too little pollen to count.

It is at once clear that the results from the present bog broadly resemble those from the Ynyslas submerged forest (Fig. 5). The clay base contains foraminifera. The lowest peat sample (24 cm.) shows a low tree/non-tree pollen ratio, and the non-tree pollen is mono-

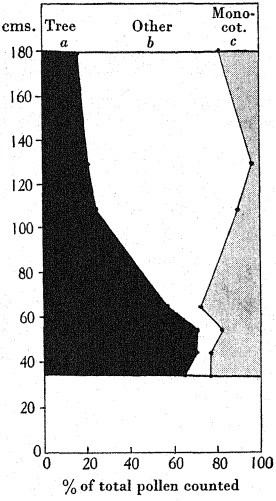

Fig. 5. Diagram of the Borth Bog profile shown in Fig. 4, expressing the relative proportions of tree pollen and of non-tree pollen of other categories.

cotyledonous. This suggests prevalent fenland, with the distant tree pollen component well marked (elm, pine, oak and hazel). The next four samples (34–54 cm.) show high tree/non-tree pollen ratios, and marked preponderance of alder and birch in the tree pollen. As at Ynyslas the alder maximum precedes that of birch and probably here also represents local fenwoods. It is not clear that here there was any local development of pinewoods. The three upper samples (100–180 cm.) show a fall to low tree/non-tree pollen ratios, and this

corresponds with the entry of *Sphagnum* spores and *Myrica* pollen in great amount. Taken with the diminishing tree pollen of the uncounted samples (180 to the bog surface) and their richness in *Sphagnum* spores, tetrads of Ericaceae, and leaves of *Myrica*, it seems clear that 100 cm. marks roughly the transition level of the bog from fenwoods to raised bog.

Conclusions

If this interpretation is correct, Borth Bog and the submerged peat at Ynyslas underwent a strikingly parallel development. This is not, of course, to say that the development was synchronous in the two cases. As to that, the evidence is lacking, though the higher pine values at the base of the Borth Bog suggest a somewhat later date than the base of Ynyslas peat, which apparently formed before pine was frequent, although it grew on the spot some time afterwards. Probably of more value as an index is the relation between the curves of *Ulmus* and *Tilia* pollen. Neither is a tree of bog or fens, and they therefore reflect in their behaviour changes in *regional* and not local conditions. It seems likely that late (Sub-Atlantic) diagrams in Britain are characterized by disappearance of *Tilia* or its subordination to *Ulmus*, in contrast with a former relative abundance. If this is so the upper part of the Borth bog diagram is definitely later than any part of the Ynyslas profile, and possibly marks the beginning of the Sub-Atlantic period.

It is, of course, particularly striking that there is no trace in the Borth bog series of the submergence which brought the coastal forest below sea-level. It should be remembered, however, that there are no records of peat composition or of pollen for the top 2 metres of the section, and it is also possible that the fen peat surrounding the present *Sphagnum* bog at Borth is the index to the submergence, and that this fen peat might be found to overlie *Sphagnum* peat. This problem would repay careful investigation.

The long developmental sequence shown by the submerged forest peat argues a long period of freedom from the marine influence under which the basal clay was deposited. Thus after the great submergence of the Boreal period, during which the North Sea was created, there must have been a considerable period before subsidence affected the sites now described. The term submergence is used here throughout, without any attempt to distinguish the eustatic and isostatic components of the movements of land and sea-level.

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EXPLANATION OF PLATE III

The submerged forest at Ynyslas, showing tree-stumps *in situ*, and exposures of peat partly covered with sand. View looking north towards the Dovey Estuary. Photo by Mr Challinor, May 1923.

GODWIN AND NEWTON—SUBMERGED FOREST, CARDIGANSHIRE

ASCORBIC ACID IN THE METABOLISM OF THE APPLE FRUIT

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F. KIDD
Low Temperature Research Station, Cambridge
AND C. WEST
Ditton Laboratory, East Malling, Kent

(With 5 figures in the text)

PRELIMINARY accounts of the progress of the investigation, the full results of which are here reported, have appeared (Zilva *et al.* 1935, 1936).

The work was the outcome of an observation that the apple contained an oxidizing enzyme (Zilva, 1934) of the nature of a phenolase (Johnson & Zilva, 1937), which could oxidize indirectly ascorbic acid *in vitro* to the non-reducing reversibly oxidized form of vitamin C-dehydroascorbic acid.

Preliminary work on English apples (Bramley's Seedling) in 1933, 1934 and 1935 and on South African apples in the winter of 1935 showed that extracts of the fruit in the early stages of its development contained only about 50 % of their vitamin C as *l*-ascorbic acid, the remainder being present in the reversibly oxidized condition as dehydroascorbic acid. Yet it had been found previously on many occasions in connexion with other investigations that in the mature fruit the vitamin existed almost entirely in the reduced form. It became therefore of primary interest to ascertain (*a*) whether the increase in the ratio of *l*-ascorbic acid to dehydroascorbic acid proceeded gradually with the development of the fruit, and (*b*) whether the equilibria observed in the extracts between the two forms of the vitamin during the development of the apple also existed as such in the cell *in vivo* and were not due to a change which took place after the disintegration of the tissues in the process of analysis. Experiments were therefore conducted during 1936 and 1937 with the

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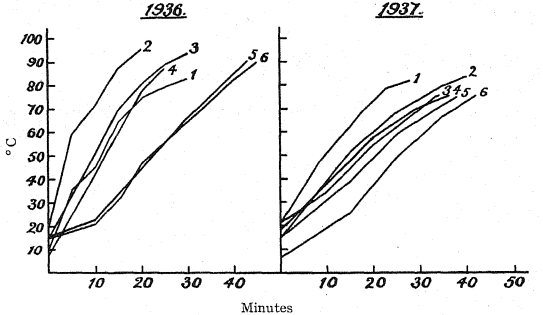
object of supplying this information. The experiments were carried out on Bramley's Seedling apples. In 1936 the fruit came off trees (half standards, Malling I rootstock) about 20 years old which were grown at the East Malling Experimental Station. In the 1937 experiments, owing to the poor crop yielded by these trees, apples from similar trees of the same plantation but on Malling VI rootstock were utilized, and, as the crop that year was a light one, the fruits were much larger on the average than those collected in 1936. In the latter year samples picked on 24 June, 17 July, 24 August, 6 October and a sample of the October pick which was stored at 3° C. for about 4 months were examined, whilst in 1937 samples collected on 16 June, 15 July, 23 August and 19 October were studied. The indophenol-reducing capacity of a number of individual fruits was determined in each batch. Two or three were used for a single determination when the fruits were very small. These determinations commenced immediately after picking and each batch was finished in about 3–4 weeks during which time the apples were stored at about 5° C. The fruit tissue including the peel was exhaustively extracted with 5% trichloroacetic acid, care being taken that the extraction did not take longer than 20–30 min., and the extracts were in the first place titrated with indophenol without previous treatment. The trichloroacetic acid extracts were then reduced with hydrogen sulphide, which was eventually expelled by a current of nitrogen, and again titrated with indophenol. The increase in the titre after reduction was taken to be due to vitamin C originally present in the extracts as dehydroascorbic acid.

In order to find out whether the oxidation took place during the process of extraction, apples corresponding to those examined raw were heated in an air-oven before extraction in order to destroy the oxidizing enzyme. The temperature of the oven was raised to 130–140° C. before the apples were introduced. When the temperature of the fruit, which was recorded on a thermometer penetrating to the centre, reached 80° C., the heating was continued for another 5 min. Owing to the fact that some reversible oxidation took place during heating, the temperature of the oven was in the case of the 1937 experiments reduced to 120–130° C., and the heating of the fruits continued for 1 min. after their temperature had reached 75° C. The gradual rise in temperature at the centre of apples of different sizes under these conditions of heating are given in Fig. 1. At several stages of the investigation the tissues of the heated apples were also examined for the presence of the oxidizing enzyme which was

invariably found to be absent. The extraction, titrations, etc., of these apples were carried out as for the raw fruit.

The reduction of indophenol is not entirely specific to *l*-ascorbic acid and although the probability that *l*-ascorbic acid was responsible for the reduction in the above experiments was great, it was hardly permissible to accept this as an established fact. Biological tests



Weight of apples:		Weight of apples:	
1. 14 g.	4. 49 g.	1. 15 g.	4. 59 g.
2. 14 g.	5. 129 g.	2. 16 g.	5. 132 g.
3. 48 g.	6. 150 g.	3. 55 g.	6. 145 g.

Fig. 1. Change in temperature in degrees centigrade at the centre of apples of different sizes during heating.

were therefore necessary to confirm this very important point. These tests with guinea pigs were carried out concurrently on each sample of apples in 1936. The results are recorded in the curves of Figs. 2–5 and provide objective evidence that the reduction of the indophenol was in the main due to *l*-ascorbic acid.

The details of the experiments are presented in Table I, which gives the ascorbic acid content per 100 g. of apple tissue of the various samples of fruit calculated from the titration values.

These results show in the first place that the ascorbic acid content of the raw Bramley's Seedling apples, as determined by the indophenol titration method, varies little with age of the apple and with the year. This is in agreement with the results obtained by us by the biological tests during the last 9 or 10 years. Furthermore, the results

TABLE I

Exp.	Date of picking	Weight of apple g. Range	Weight of apple g. Mean	Raw fruit: *l*-Ascorbic acid content (mg. per 100 g. of fruit)	Raw fruit: Total vitamin C content (mg. per 100 g. of fruit)	Raw fruit: Average % of total vitamin C present as *l*-ascorbic acid	Heated fruit: *l*-Ascorbic acid content (mg. per 100 g. of fruit)	Heated fruit: Total vitamin C content (mg. per 100 g. of fruit)	Heated fruit: Average % of total vitamin C present as *l*-ascorbic acid
1	24. vi. 36	5–23	12	11·16	15·48		4·68	22·68	
				7·92	19·20		9·00	18·60	
				12·96	21·60		6·84	12·24	
				1·44	14·04		2·25	6·48	
				11·52	20·16		7·56	17·28	
				5·04	20·52		5·04	7·56	
				—	—		7·56	11·16	
				8·34	18·50	45·1	6·13	13·71	44·7
2	17. vii. 36	29–64	38	2·52	9·36		8·64	11·52	
				1·08	9·72		3·24	7·20	
				5·76	14·76		12·60	19·20	
				3·60	26·64		5·76	12·60	
				—	—		10·08	17·64	
				—	—		7·80	20·88	
				—	—		6·60	14·40	
				—	—		9·00	18·00	
				—	—		10·80	21·96	
				—	—		7·80	15·60	
				3·24	15·12	21·4	8·23	15·90	51·7
3	24. viii. 36	52–106	70	18·72	23·40		14·40	18·00	
				11·88	18·36		11·52	16·20	
				17·64	19·08		14·40	19·80	
				19·08	21·96		12·60	21·24	
				0·00	14·40		7·80	12·60	
				7·80	21·00		18·00	21·60	
				11·52	15·00		16·92	13·20	
				12·38	19·03	65·1	13·66	17·52	78·0
4	6. x. 36	71–217	124	20·88	20·88		12·24	17·28	
				18·72	20·16		20·88	20·88	
				11·88	14·40		6·12	14·04	
				15·84	16·92		10·08	15·00	
				14·40	18·00		10·08	16·20	
				7·56	14·40		—	—	
				18·00	18·00		—	—	
				15·33	17·54	87·4	11·88	16·68	71·2
5	6. x. 36 (and stored till 2. ii. 37)	72–168	110	20·88	20·88		16·56	20·16	
				15·12	16·92		15·48	21·60	
				21·24	24·12		15·60	19·80	
				25·92	26·64		10·08	19·80	
				26·64	27·36		23·40	27·00	
				18·72	19·80		19·80	20·52	
				29·52	29·88		10·08	15·12	
				17·64	17·64		9·36	11·88	
				17·64	22·68		10·80	14·40	
				20·88	25·92		8·28	12·60	
				20·16	20·52		—	—	
				21·31	22·94	92·8	13·94	18·29	76·2

TABLE I (*continued*)

Exp.	Date of picking	Weight of apple g. Range	Weight of apple g. Mean	Raw fruit: *l*-Ascorbic acid content (mg. per 100 g. of fruit)	Raw fruit: Total vitamin C content (mg. per 100 g. of fruit)	Raw fruit: Average % of total vitamin C present as *l*-ascorbic acid	Heated fruit: *l*-Ascorbic acid content (mg. per 100 g. of fruit)	Heated fruit: Total vitamin C content (mg. per 100 g. of fruit)	Heated fruit: Average % of total vitamin C present as *l*-ascorbic acid
6	16. vi. 37	8–18	12	4·68	16·92		2·88	12·60	
				7·92	19·80		2·88	9·72	
				4·68	15·84		1·80	11·16	
				5·76	18·72		3·60	12·60	
				2·88	15·12		0·72	9·72	
				9·00	19·08		2·16	16·20	
				6·12	16·56		2·16	14·76	
				12·60	21·24		5·04	11·52	
				11·16	20·16		2·52	16·20	
				9·72	18·36		1·44	11·88	
				5·76	15·12		3·24	11·16	
				7·56	16·56		3·24	13·32	
				10·44	23·76		4·68	10·80	
				7·92	18·36		4·32	15·84	
				7·59	18·26	41·6	2·91	12·68	22·9
7	15. vii. 37	41–86	59	8·64	14·04		7·20	17·28	
				10·44	19·44		5·04	16·20	
				12·24	20·88		9·36	19·08	
				12·96	19·08		7·20	32·40	
				12·96	24·12		9·36	22·80	
				19·08	30·96		9·36	24·48	
				9·60	16·92		6·48	18·00	
				12·27	20·78	59·0	7·71	21·46	35·9
8	23. viii. 37	107–175	138	14·76	16·56		6·12	13·68	
				14·04	16·56		9·60	18·00	
				6·48	17·28		4·68	15·12	
				15·48	16·92		17·64	23·76	
				19·08	19·80		10·08	16·20	
				18·00	20·52		4·32	6·84	
				25·92	27·00		6·84	8·28	
				12·60	14·76		6·48	13·32	
				14·76	16·92		7·20	12·60	
				—	—		6·84	11·16	
				—	—		10·80	16·80	
				—	—		6·48	11·52	
				15·68	18·48	84·8	8·09	13·94	58·0
9	19. x. 37	119–209	171	14·40	17·64		9·00	14·40	
				17·64	19·44		13·32	16·56	
				12·96	13·68		14·04	18·72	
				22·32	22·32		12·60	17·28	
				19·08	19·08		12·60	21·24	
				16·92	16·92		6·48	14·40	
				22·68	22·68		13·32	16·92	
				18·00	18·82	95·6	11·62	17·07	68·1
10	8. vii. 35	24–36	31	7·19	18·95		7·54	20·53	
				5·26	18·95		6·31	13·33	
				5·61	19·65		2·28	15·70	
				6·31	13·51		4·82	17·72	
				9·12	18·07		6·67	20·16	
				7·19	14·56		7·19	12·62	
				11·40	24·91		3·68	7·36	
				13·16	17·89		10·00	16·31	
				8·42	21·05		7·89	11·40	
				10·35	26·14		8·77	18·95	
				8·40	19·37	43·4	6·52	15·41	42·3

of the biological tests which were carried out simultaneously with the titrations during 1936 agree well with the average values of ascorbic acid of the respective samples of apples calculated from the figures of the indophenol titrations after reduction with hydrogen sulphide. Unfortunately, owing to the limited amount of material, it was not possible to test biologically the raw apples picked in July and in August. The heated apples of these samples were, however, assessed in this way. The response of the experimental animals on the various doses of raw apple were found to be very similar to those which received corresponding doses of pure ascorbic acid (Fig. 2). It follows, therefore, that under the above experimental conditions the indophenol titration figures are an accurate index of the ascorbic acid content of the tissue extracts. This is also true of the figures for the heated apples. Only on one occasion with fruit picked in August, 1936, did the response of the animals to the heated fruit fall somewhat below that indicated by the titration values. This insignificant deviation, if real, was in all probability due to the presence of small quantities of reductic acid formed during the heating, a substance capable of reducing indophenol but not possessing antiscurvy properties.

Examining the results obtained with the raw apples first, it is seen that there was a gradual increase in the percentage of vitamin C present as ascorbic acid in the extracts of the apples, from 45·1 in the June sample to 92·8 in the October sample (stored until 2 Feb. 1937) in 1936 and from 41·6 in the June sample to 95·6 in the October sample in 1937. Only the figures of the 1936 July sample of raw apples (Table I, **2**) failed to show the progressive increase above the next youngest sample. For this experiment only a very few apples were available and moreover, as the figures for their total vitamin C content show (only 15·1 mg. per 100 g. of tissue), they were not typical fruits. It is significant also that the heated apples of this sample, of which a greater number were analysed, show the characteristic rise in the percentage of the reduced form of the vitamin.

As in the raw apples, the heated fruit, with the exception of the

Legend to Fig. 2

┆ =Commencement of dosing. ↓ =Onset of clinical symptoms of scurvy. *C*=Chloroformed. +=Died. *N*=Normal. *VSS*=Very slight scurvy. *SS*=Slight scurvy. *S*=Scurvy. *P*=Pneumonia.

Fig. 2. Growth of guinea pigs receiving doses of *l*-ascorbic acid above, and apple pulp, Exp. **1** (24. vi. 36) below.

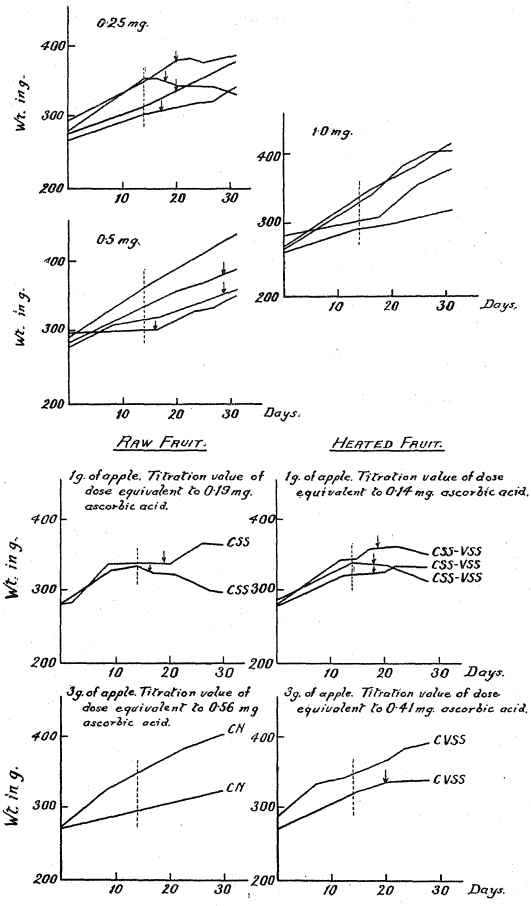
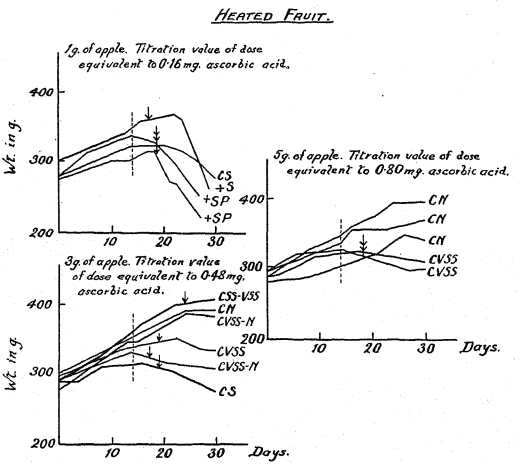

L-Ascorbic Acid.
0·25 mg.
1·0 mg.
0·5 mg.
Wt. in g.
400
300
200
10
20
30
Days.
Raw Fruit.
Heated Fruit.
1g. of apple. Titration value of dose equivalent to 0·19 mg. ascorbic acid.
1g. of apple. Titration value of dose equivalent to 0·14 mg. ascorbic acid.
CSS
CSS
CSS-VSS
CSS-VSS
CSS-VSS
3g. of apple. Titration value of dose equivalent to 0·56 mg ascorbic acid.
3g. of apple. Titration value of dose equivalent to 0·41 mg. ascorbic acid.
CN
CN
CVSS
CVSS

Fig. 2.



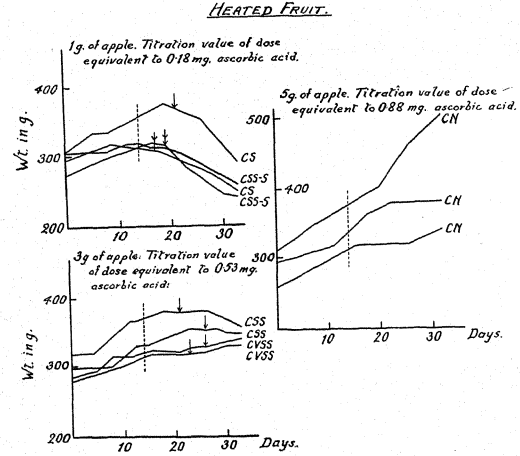


Fig. 3. Exp. **2** (17. vii. 36) above, and Exp. **3** (24. viii. 36) below. See Fig. 2 for explanation of symbols.

1936 October sample (Table I, 4), in spite of a certain loss in the total content, showed a progressive rise in the concentration of vitamin C present as ascorbic acid with the development of the

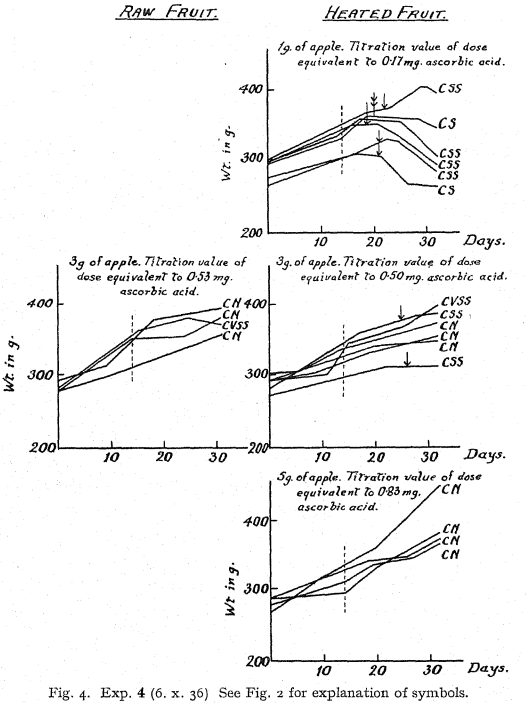


Fig. 4. Exp. 4 (6. x. 36) See Fig. 2 for explanation of symbols.

apples. Of particular interest, considering the drastic methods employed, is the fact that the relative quantities of ascorbic acid and dehydroascorbic acid present in the various samples of apples in 1936 were of a similar order to those found in the corresponding

samples of the raw fruit. As the oxidizing enzyme was destroyed in the heating and the time taken to prepare the extracts was short, it may be concluded that, at least, the best part of the dehydroascorbic

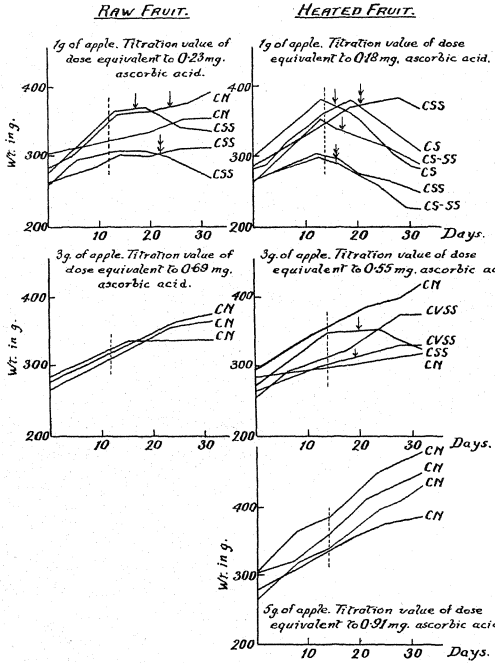


Fig. 5. Exp. **5** (6. x. 36, then stored till 2. ii. 37).
See Fig. 2 for explanation of symbols.

acid found in these extracts was not formed in the manipulation. Nor is it at all probable that by a mere coincidence it was formed in every case by atmospheric oxidation during the heating in similar

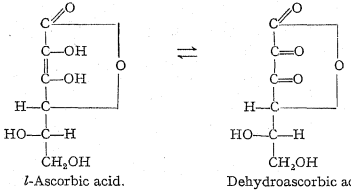
proportions to those found in the extracts of the raw apples, especially as this phenomenon was also observed in 1935 (Table I, **10**).

The results of the 1937 experiments resemble those of 1936 in that they also show a progressive diminution in the dehydroascorbic acid content of the heated as well as of the raw apples as the fruit develops. In 1937, however, the ratio of dehydroascorbic acid to ascorbic acid in the heated fruit was higher than that in the corresponding raw apples. Presumably, in this season, some reversible oxidation of ascorbic acid took place during the heating of the experimental material. This cannot be explained at present. The progressive diminution with the age of the fruit in the content of the dehydroascorbic acid of the extracts of the heated apples nevertheless suggests that in this case also some of it must have been preformed *in vivo*.

The general evidence seems so far to support the view that dehydroascorbic acid is present in the living tissue of the young apple and that it tends to diminish as the fruit approaches maturity.

Discussion

The bearing of the above results on the metabolism of the apple cannot be discussed without first taking into consideration the mechanism of the reversible oxidation of *l*-ascorbic acid. Although some oxidizing reagents break irreversibly the carbon chain of ascorbic acid with the production of oxalic and *l*-threonic acids, others such as iodine, certain indophenols, etc., have a less drastic effect on the molecule. Thus the latter treatment simply removes two hydrogen atoms, without even opening the ring, yielding a product of oxidation—dehydroascorbic acid—to which the hydrogen atoms can be restored by chemical reduction such as treatment with hydrogen sulphide according to the following scheme:



l-Ascorbic acid. Dehydroascorbic acid.

The reversible oxidation of *l*-ascorbic acid can also be effected biologically. Thus in the animal kingdom dehydroascorbic acid is reduced to *l*-ascorbic acid *in vivo* and also by certain tissues *in vitro*. The equilibrium in this instance is therefore such that only the reduced form of vitamin C can be detected (Johnson & Zilva, 1934; Kellie & Zilva, 1935, 1936).

Many plant tissues oxidize *l*-ascorbic acid to dehydroascorbic acid *in vitro* and it is now known that this oxidation can be brought about by at least three distinct enzyme systems present in plants. Szent-Györgyi (1928) has shown in the first place that peroxidase is capable of effecting this reversible oxidation. Later (1930, 1931) he described another enzyme—an aerobic oxidase—present in cabbage, which oxidized *l*-ascorbic acid directly. This enzyme has since been found in a number of other plants. Finally Zilva (1934) demonstrated that apple juice was capable of oxidizing reversibly *l*-ascorbic acid. This, however, was shown later by Johnson & Zilva (1937) to be due to the indirect action of a polyphenolase and not to the direct action of an ascorbic acid oxidase similar to that present in the cabbage.

Johnson & Zilva extracted an enzyme from the apple tissue residue, left after expressing the juice which oxidized mono- and polyhydric phenols to their corresponding quinones. Ascorbic acid could only be dehydrogenated by this enzyme in the presence of catechol, phenol or pure apple juice. The ascorbic acid was, therefore, oxidized not directly as in the case of the cabbage enzyme but by the quinones formed through the action of the polyphenolase on the phenols. Pure juice alone did not oxidize ascorbic acid. The activity of the crude juice was no doubt due to the presence in it of suspended material with which the polyphenolase is associated.

It would therefore appear that in all probability the enzyme involved in the *in vivo* oxidation of ascorbic acid in the apple is not a direct ascorbic acid oxidase but a polyphenolase and that the ascorbic acid present in the fruit acts as a carrier between this and another system. If this be so, it would seem that the change in the equilibrium of the two forms of *l*-ascorbic acid is a function of some metabolic process connected with the growth of the apple. Work now in progress promises to throw further light on this interesting problem.

SUMMARY

1. Vitamin C is present in the apple, both as *l*-ascorbic acid (reduced form) and as dehydroascorbic acid (reversibly oxidized form).

2. The total quantity of vitamin C present in these two forms remains constant, per unit of fresh weight, throughout the growth of the apple.

3. There is, however, a change in the relative proportions of the two forms. As the fruit approaches maturity, the proportion of *l*-ascorbic acid increases and that of dehydroascorbic acid decreases.

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THE EFFECT OF *LOPHODERMELLINA MACROSPORA* (HARTIG) TEHON ON LEAF-ABSCISSION IN *PICEA EXCELSA* LINK.

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(With Plate IV and 2 figures in the text)

It is a well-known character of the spruces that their leaves are shed very rapidly from a cut twig which has been allowed to dry. An exception follows the infection of *Picea excelsa* by *Lophodermellina macrospora* (Hartig) Tehon, when needles showing the (so-called) α-form of the disease remain firmly attached after the uninfected ones have been shed. This phenomenon has been remarked upon by a number of authors, while explanations have been advanced by Mer (1910) and Neger (1911). These explanations appeared inadequate to the writers, who undertook a detailed anatomical and experimental study both of the cause of the retention of the infected needle, and of the mechanism of abscission in the normal leaf, a clear understanding of which is essential for solution of the main problem.

I. The leaf-abscission mechanism

The mechanism of leaf abscission in *Picea excelsa* may be conveniently studied by allowing a cut branch to dry at room temperature. In the course of a few days, the time depending on the temperature and humidity of the room, the leaves all become detached at a gentle touch or shake. Behrens (1875) originally pointed out that drying upset the structural relationships of the tissues at the point of contact of the leaf and cushion, causing a greater contraction of the tissues of the leaf base than of those of the adjoining cushion. This ultimately resulted in their separation. Neger (1911) elaborated this conclusion, maintaining that the leaf fall was almost exclusively a physical phenomenon. Later Neger, in collaboration with Fuchs (1915), made a fuller anatomical study of the tissues involved in abscission. This account is substantiated by the present examination, which has also revealed a number of points unrecorded by previous investigators.

The peg-like cushion upon which the leaf is borne is very similar in structure and appearance to the branch from which it projects. Immediately above the cushion is a clear, hyaline band which completely encircles the base of the leaf. This is the hyaline layer. Reference to the longitudinal section through leaf and cushion, shown in Pl. IV, fig. 2, will illustrate the following brief anatomical account. The vascular bundle is continuous in cushion and leaf. Xylem and phloem, embedded in parenchymatous tissue, are enclosed in an endodermis consisting of long narrow cells whose outer walls are slightly thicker than the inner. In the leaf the vascular bundle contains a small group of sclerenchymatous cells, which are absent from the cushion.

Surrounding the vascular bundle in the cushion and branch is the chlorenchyma, of uniform width except at the tip of the cushion, where an increase in this tissue results in a dome formation. Outside this layer is the phellogen, a broad band of cork cells, and finally the well-developed epidermis with thick cuticle.

The dividing line between cushion and leaf tissues is a layer, never exceeding 2 cells in width, composed of small, irregular cells with comparatively thick walls. Neger named these the "tooth cells". A point hitherto unrecorded is that, on staining with Sudan III, a red coloration resistant to alcohol appears in the cuticle and in the walls of the tooth cells, indicating the presence there of cutin-like material. Above the tooth cells is the hyaline layer which consists of much larger cells with very thick walls and small lumina. These are of the stone-cell type, having lignified walls pierced by prominent pit canals. Under polarized light, striations in the walls are clearly visible and, since the cells are elongated parallel to the longitudinal axis of the leaf, the striations are most prominent in this direction. Thus hygroscopic contractions of the walls will be effected at right angles to the leaf axis. In each cell of the hyaline layer protoplast and large nucleus are clearly visible. In the writers' opinion, the hyaline layer should be regarded as part of the hypodermis, to which it bears a very close resemblance both in structure and mechanical function. Thus the entire leaf is enclosed within a sheath of sclerenchyma except at the entrance of the vascular track. The tooth cells, too, appear as a modified continuation of the epidermis.

A number of experiments carried out by Neger (1911) demonstrated clearly that water loss was the factor activating the leaf-fall mechanism. He regarded the whole process as an almost purely physical phenomenon depending upon the water content of the leaf. His

experiments have been repeated by the present writers and, in general, confirmed. In brief, it has been shown that both living and dead (killed by chloroform) twigs quickly cast their leaves if placed under drying conditions, while similar twigs kept in a damp atmosphere retain their leaves indefinitely. Likewise twigs with leaves carefully vaselined or waxed will retain their leaves although exposed to drying conditions. In such experiments the period of time elapsing before abscission takes place depends upon the drying method employed, varying from 10 to 15 min. in 100° C. oven to 48 hr. in a desiccator. Under room conditions, a period of 3–14 days passes before abscission, while twigs kept in a damp chamber have been shown to retain their leaves after 5 months. From these experiments, it would appear that abscission is determined simply by the loss of a certain quantity of water. However, estimations by the present writers of the actual amount of water lost by the leaf before abscission takes place have given very variable results under different conditions. The water content of healthy spruce leaves is 55–60 % of their fresh weight. Neger (1911) found that, in twigs placed in a desiccator, the leaves fell when the water loss exceeded 20 %. Under room conditions, the writers found a water loss of 30–40 % was necessary for abscission. Also it was discovered that leaves from twigs kept in a damp chamber for 5 months had lost 35 % of their fresh weight without being shed. It is clear from these and other experiments that the exact amount of water lost from the needle is not the significant factor in leaf abscission. Rather is it the rate of water loss and particularly the rate of water loss from the abscission mechanism, the hyaline layer. In cases of rapid drying, it does not follow that water loss from the hyaline layer is proportional to that from the leaf as a whole. Abscission depends upon the separation of the hyaline layer from the tissues of the cushion. The hyaline layer is, by its structure, capable of large hygroscopic contraction at right angles to the leaf axis. The tissues of the cushion, on the other hand, have no such specialized development, so that any contraction in this neighbourhood would be non-hygroscopic. Further, the cushion tissues are protected against rapid water loss by the strong development of the periderm. Thus, in extreme drying conditions, the hyaline layer will lose water much more rapidly than the protected cushion and consequently abscission will result. On the other hand, in a very moist environment where the water loss is a gradual process, the hygroscopic apparatus will not be activated. Thus there will be no significant difference between the hygroscopic contraction of the hyaline layer and the shrinkage of the

dead cushion tissues in specimens which have been kept for a long period in a damp chamber. Consequently, in spite of a considerable water loss, abscission will not take place. It appears, then, that it is the relative rate of water loss which is the factor determining leaf abscission; the greater the water loss of the hyaline layer in relation to that of the cushion tip, the more rapidly will leaf fall be brought about.

When the greater contraction of the hyaline layer causes a fracture between leaf and cushion, the leaf remains attached by means of the fragile vascular strand. The final separation will rarely occur until a touch or shake snaps this connexion.

In the writers' opinion, Neger's (1911) explanation of leaf abscission, as a purely physical phenomenon dependent upon water loss from the leaf, does not give an accurate picture of normal leaf fall. The drying conditions necessary to bring about the abscission of a normal, living leaf are unnatural, and, were such conditions to exist in nature, the probable result would be complete defoliation of the tree. There is no question of an abscission mechanism being formed in old needles preparatory to their fall since, as Neger has shown, the hyaline layer is laid down very early in the leaf's development. Some other explanation must be sought. Obviously, the abscission mechanism is prevented from functioning in both living and dead needles by their high water content. In the living needle, the vascular system provides sufficient water to counteract drying agencies in the tree's normal environment. Reduction in the water content of the leaf can most readily be achieved by blocking the vascular track. Examination of the cushions, from which leaves have recently become detached, has invariably shown a deposit of a brownish substance completely filling the bundle and the surrounding tissues. This substance is absent from the cushions of living needles. Experiments upon its formation were conducted by killing leaves, still on the tree, with chloroform. Periodic inspection showed that after 2 days the brown substance appeared in the phloem. In later samples the substance had spread throughout the entire vascular tract in the cushion. This occurred some days before abscission took place. As to the nature of the brown substance the only positive reaction to microchemical tests was a red coloration with phloroglucin and hydrochloric acid, suggesting the presence of the complex known as "wound gum". The substance is shown filling the vascular bundle in Pl. IV, fig. 1. The presence of this "wound gum" in the vascular bundle of the cushion is capable of different interpretations. No absolute proof is available to show that blocking of the vascular system is a

necessary precursor of normal leaf fall but, in the writers' opinion, this appears to be highly probable. Such a stoppage of the water supply would be most effective in bringing into action under normal environmental conditions the abscission mechanism.

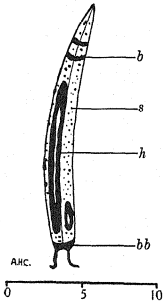
II. *LOPHODERMELLINA MACROSPORA* (HARTIG) TEHON

Hartig (1874), who originally described this parasite of *Picea excelsa*, distinguished three forms of the disease chiefly by the rate of production of hysterothecia on infected needles. He remarked, too, that in two of the forms the leaves remained attached to the twig, while in the third they fell from the tree. Prillieux's (1897) account of the disease is essentially that of Hartig. A thorough investigation by Mer (1910) revealed a number of points which apparently escaped Hartig. Mer distinguished two forms of the disease, form α in which the infected leaves remain attached to the twig, and form β where the needles fall from the tree. Infected needles of form α have a black basal ring, whereas those of form β have none. In α-form leaves, the mycelial growth is very rapid, while in β-form leaves the fungous development is so much slower that the whole leaf may not be involved until long after infection. Mer also showed that the type of disease depended upon the time of infection and the constitution of the attacked needles and not upon physiological differences in the fungus.

The material used in this investigation, Mer's α-form of the disease, was collected by one of us (A.H.C.) from a 30 years old plantation of *Picea excelsa* at Ballindalloch, Banff. It agreed in all respects with Tehon's (1935) diagnosis of *Lophodermellina macrospora* on *Picea Abies*. *Picea Abies* Karst. is a synonym of *Picea excelsa* Link. The fungus, under the name *Lophodermium macrosporum*, has been recorded in Scotland previously by Watson (1917). Only those aspects of the life history of the fungus which have a bearing upon the retention of the needles in the α-form of the disease will be dealt with here.

The distinctive feature of leaves showing the α-form of the disease is a black ring around the base in the region of the hyaline layer. Occasionally, a leaf is encountered showing one or more black rings close to the tip as in Text-fig. 1. As these rings represent the only morphological difference between the α- and β-forms of the disease, it was to be expected that the explanation of the retention of the needle should be sought in them. A longitudinal section through the

leaf base (Pl. IV, fig. 1) shows that the black ring covers the whole region of the hyaline layer. Mer (1910) attributed its formation to a blackening of the cells by a deposition of resins and tannins to combat the activity of the fungus. He maintained that this impregnation prevented the contraction of the leaf base and hence abscission could not take place. Neger (1911) described the black ring as formed of dark mycelial masses in the cells of the hyaline layer. This mycelial aggregation, he said, prevented contraction of the layer and resulted in the retention of the needle.



Text-fig. 1. Leaf of *Picea excelsa* infected by *L. macrospora*, showing characteristic external appearance of the α-form of the disease: thus *b*, black line; *bb*, basal black line; *h*, hysterothecium and *s*, substomatal sclerotium.

In very thin sections the writers have observed large, dark brown hyphae in the lumina of the hyaline layer cells, but the chief cause of the blackening is a dark brown pigment produced by the hyphae which stains both cell walls and contents (Pl. IV, fig. 1). The dark hyphae can be studied more readily in the black lines which occur towards the tip of the needle. Here they are seen to be brown and many-branched with an average diameter of 3 μ. They are aggregated into a thin, pseudoparenchymatous partition across the leaf generally about 20 μ in thickness but which becomes much more substantial in the vascular bundle. The dark pigment produced by these hyphae, which is chiefly responsible for the substantial appearance of the black ring, spreads over a much wider area, infiltrating the chlorenchyma and staining the numerous starch-grains to be found there. Apart from the black rings, these large, brown hyphae form the base and

cover of the hysterothecium, the walls of the pycnidia and the entire body of the substomatal sclerotia. The hyphae, varying from 2 to 8 μ in diameter with an average of 4 μ, form a melenchymatous (Hilitzer, 1929) or pseudocollenchymatous (Tehon, 1935) tissue in which the interstices between the cells are filled by a deposit of brown pigment. Of chief interest to the present study are the substomatal sclerotia, which completely block the stomata in leaves showing the α-form of the disease. Careful examination of the whole leaf shows that every stoma is occluded by a black, pseudocollenchymatous plug. Killian & Likhité (1924) and Likhité (1926) regard these bodies, at least in some cases, as primordia in which fertilization takes place prior to the development of the hysterothecium. The great number of these substomatal sclerotia makes this explanation unlikely in every case. Hilitzer (1929) maintains that they are the first-formed mycelium from the germinating spore and that they serve as a nidus for the spread of the mycelium in each individual infection. This view would require a separate infection for each stoma in the leaf and is, therefore, improbable. Tehon (1935) has indicated objections to the previous interpretations of the nature of the substomatal sclerotium and suggests that it has arisen from, rather than given rise to, the mycelium in the leaf. The large, dark hyphae of the substomatal sclerotium are quite distinct from the internal mycelium which is hyaline and fine with a diameter of 0·6–1 μ. In the writers' view, the only function which can be attributed to the substomatal sclerotia is that of a mechanical blocking of the stomata. Anatomical studies have failed to show anything suggestive of a young pycnidium or hysterothecium in the position of a substomatal sclerotium. Its structure is extremely simple, consisting of a pseudocollenchymatous plug from which the brown pigment has diffused and stained the stomatal cells.

III. THE RETENTION OF INFECTED NEEDLES

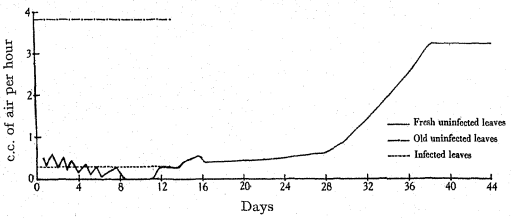
It has been shown above that leaf abscission in the normal spruce needle is brought about by a water loss from the needle considerably in excess of that from the peg. The retention of the infected needles in the α-form of the disease must be due either to the inability of the hyaline layer to contract or to the prevention of water loss by the mycelium in the leaf. The former explanation was advanced by Mer (1910) and Neger (1911). Mer believed that the blackening of the hyaline layer was due to a deposit of resins and tannins, while Neger

attributed it to the presence of dark masses of mycelium. Both apparently held that the presence there of resin or hyphae, functioned mechanically in preventing the contraction of the hyaline layer, but neither author presented experimental data in support of the hypothesis.

It seemed of first importance to discover by direct measurement whether contraction was possible for the blackened hyaline layer. Consequently, needles were selected at random from twigs bearing both infected and uninfected needles of the same age. Measurements were made of the hyaline layer in healthy needles and of the black line at the same level in infected needles, under the low power of the microscope using a micrometer. Inspection showed that the basal widths were in agreement within each group and that the mean basal width of the infected leaves was less than that of the normal. This difference amounted to 5·1 % of the basal width of the normal, uninfected leaf. In a second experiment, infected leaves were attached to a glass slide and the width across the black line noted. The leaves were then thoroughly soaked in water and again measured, when an enlargement of 6·2 % was recorded. Finally, on rapidly drying out the needles, a decrease of 1·4 % below the original width was obtained. Thus, during the experiment, a hygroscopic movement amounting to 7·6 % of the original width of the hyaline layer had taken place despite the presence of the fungus. A similar experiment carried out with fresh leaves attached to a small piece of twig showed that abscission took place at room temperature on the fifth day following a contraction of 5–6 % of the original basal width. Therefore, so far from the hyaline layer in infected needles being unable to contract, it is capable of a hygroscopic movement sufficient to bring about abscission in normal, uninfected leaves.

The alternative hypothesis that water loss from infected leaves is reduced by the presence of the fungus was then examined. In a heavily cuticularized leaf, such as that of the spruce, water loss takes place principally through the stomata and thus, external conditions being constant, an index of the rate of water loss can be obtained by measuring the amount of air which can be drawn through the stomata. For this purpose an inverted porometer was devised. In this apparatus the bell consisted of a small thistle funnel, fitted with a thin slice of cork through which the needles were stuck perpendicularly, one-half in the bell and the other half protruding. The leaves and cork were most carefully sealed in with paraffin wax and the bell attached to the usual water container, bubbler and siphon. In the

completed apparatus, the only points at which air could enter the system were the exposed parts of the leaves under test. Air was drawn through the leaves by means of a water siphon with a drop of 3 ft. and its passage recorded by bubble counts. Three of these porometers, each containing nine needles, were set up and operated under the same conditions; two of the systems contained uninfected needles and one infected needles. Of the uninfected leaves, one lot were fresh and the other old and air-dry. The old, uninfected needles were of the same age as the infected ones, having been shed from a specimen twig to which infected leaves still remained attached. Calibration of the bubblers was carried out by measuring in ccs. the water displaced by 1000 bubbles in each apparatus.



Text-fig. 2. Constant rates of passage of air were observed for both old, infected and uninfected leaves, amounting respectively to 0·3 and 3·8 c.c. per hour. Fresh, uninfected leaves showed a periodic variation for the first 8 days, which was apparently dependent upon rhythmic stomatal movements. In the next phase lasting about 2 days, no air passed through the system due presumably to the complete closure of the stomata. Thereafter, the rate of passage of air gradually increased until the end of the experiment.

The record of results is shown in Text-fig. 2, expressed as the rate of passage of air in c.c. per hour over a period of 44 days. The old, uninfected needles gave a constant reading of 3·8 c.c. per hour for the first 12 days, while the infected needles recorded 0·3 c.c. per hour for the same period, after which the readings were discontinued. The fresh leaves showed a periodic variation, apparently dependent upon rhythmic stomatal movements, for the first 8 days. Then came a period of complete closure lasting over 2 days which was followed, in turn, by a gradually increasing rate of the passage of air until the end of the experiment. It is clear from the graph that infected needles present much more resistance to the passage of air (and presumably water-vapour) than the uninfected needles. This phenomenon is

undoubtedly brought about by the blocking of the stomata by the substomatal sclerotia. The rate of evaporation from the leaves depends primarily upon the temperature and humidity of the environment; but, whatever the environment, it is clear from the porometer experiment that the physical barrier provided by the substomatal sclerotia will always greatly reduce that rate. This slower water loss from the infected leaves means a slower contraction of the hyaline layer. Thus is brought about a condition similar to that of normal needles kept in a damp chamber; the leaves remain attached to the twig, since there is not sufficient difference in the contraction of leaf and cushion to cause abscission.

From the point of view of damage to the tree, it is of no importance whether the leaves remain upon the tree or not, since they are soon killed and their separation from the tree completed by the deposit of "wound gum" in the cushion. However, the retention of the infected needles would appear to be advantageous to the fungus, particularly in making possible a more effective dispersal of the spores among the uninfected leaves.

Virtually, the leaf is converted by the fungus into a sclerotium-like body whose rind, consisting of the epidermis of the leaf, is rendered complete by the blocking of the stomatal apertures (substomatal sclerotia), and by the provision of mycelial plugs (black rings) at the base and, sometimes, near the tip of the needle. The medulla consists of the numerous hyaline hyphae of the fungus ramifying through the internal tissues of the leaf, the cells of which are packed full of starch grains. Thus the infected needle serves the fungus as a kind of sclerotium, by affording the mycelium protection from desiccation and by preserving within it a food supply for the further development of the fungus.

Summary

The abscission mechanism of the spruce needle depends upon the hygroscopic contraction of the hyaline layer following water loss from the leaf. The decisive factor is not the actual amount of water lost by the leaf, but the comparative rate of water loss from the hyaline layer and the adjoining tissues of the cushion.

In the α-form of the disease produced by *Lophodermellina macrospora* on *Picea excelsa*, the infected leaves, although dead, remain firmly attached to the tree. Mycelial plugs, in the form of substomatal sclerotia and black plates, convert the leaf into a kind of sclerotium.

Such leaves have a very greatly reduced rate of water loss, and in consequence the contraction of the hyaline layer is not sufficient to activate the abscission mechanism.

The writers desire to thank Dr Malcolm Wilson and Mr D. Mackenzie for their assistance in obtaining supplies of the fungus.

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EXPLANATION OF PLATE IV

Fig. 1. Longitudinal section infected needle and cushion. The hyaline layer has been blackened by the hyphae and brown pigment. Note the deposit of "wound gum" in the vascular bundle of the cushion. Unstained.

Fig. 2. Drawing of longitudinal section of uninfected leaf and cushion to illustrate the abscission mechanism. Description in text, p. 359.

Fig. 1

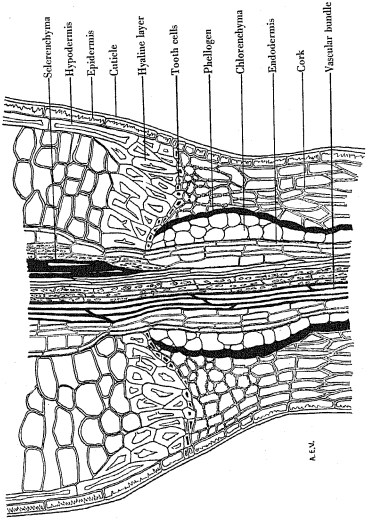
Sclerenchyma
Hypodermis
Epidermis
Cuticle
Hyaline layer
Tooth cells
Phellogen
Chlorenchyma
Endodermis
Cork
Vascular bundle
A.E.M.

Fig. 2

A MULTIPLE-ENTRY PERFORATED-CARD KEY WITH SPECIAL REFERENCE TO THE IDENTIFICATION OF HARDWOODS

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(With 1 figure in the text)

THE vast majority of the identification keys used by workers in biological fields are constructed on the dichotomous pattern, in which the user has to decide between successive pairs of mutually exclusive alternatives. Such keys may be presented in various forms (e.g. Chalk & Rendle, 1929; Dadswell & Burnell, 1932; Record, 1934), but all are subject to a serious disadvantage in that, as Bianchi (1931) has pointed out, at each pair of alternatives, "one is obliged to choose, even if the object in hand does not allow one to choose with certainty, between the features in question". The case may be imagined of two woods easily distinguishable on, say, the arrangement of the vessels or the type of ash left on burning a splinter, but which in the key must be separated at some stage on a feature which is not nearly so distinctive. This is unavoidable when it is only possible to use the diagnostic features in a specified order, and it is clear that the ideal key will not impose such a condition. The idea of multiple-entry keys, allowing the features to be considered in any order, is not new (Bianchi, 1931; Swain, 1927), and several forms have been suggested.[1] In weighing the merits of such keys it should be remembered that besides accuracy in identification, the following attributes are desirable: (i) simplicity in operation, and (ii) an elasticity that will allow the inclusion of new species in the key without undue rearrangement of the existing structure. The perforated-card key described in this note has been in use at Princes Risborough for nearly two years, and is proving satisfactory on all counts.

[1] The principle was suggested in a periodical about 1928, but the writer has been unable to trace the reference; Record (1932) has also referred to the possibility of using a form of selector card index for identifying timbers.

Fig. 1. Layout of card for multiple entry key. Features 1, 3 and 7 are shown notched. Actual size of card 5 × 8 in.

Of the various perforated-card systems on the market there is reason to believe that almost any would be satisfactory, but the one described (the Paramount Sorting System[1]) has the advantage of simplicity, and requires the minimum of apparatus. The cards bear a series of marginal perforations, each one representing some diagnostic feature (Fig. 1), and a separate card is used for each genus or species to be included in the key. The presence of a feature is recorded by notching the appropriate hole with a pair of clippers, and the absence by leaving the hole unnotched (Fig. 1). In consequence, when a thin steel rod is pushed through the complete pack of cards through the holes representing a particular feature, and the pack gently shaken, all the cards of species showing the feature fall out. In identifying a timber this process is repeated on the cards that fall out until only one is left, and this should bear the name of the timber to be identified.

Wherever possible features are defined so that the minimum of notching is necessary (e.g. in Fig. 1 the statement "rays homogeneous" calls for less notching than would "rays heterogeneous"). Where a species presents a borderline condition of any feature it is found safer to notch the hole; where a feature varies in different specimens of a species (e.g. indefinite growth rings, sporadic septation of fibres), an ink line is marked over the unnotched margin of the hole. In some cases it is desirable to use two cards for a single species. These precautions are only necessary, however, to obtain a complete description; in using the key only features that are well defined in the specimen under examination would be employed.

It may be observed that each card bears a detailed and readily appreciated description of the species, and the pack may be used to furnish lists of species showing particular features. Another great advantage of the card key is that the country of origin may be made a point of entry.

TABLE I. *List of characters used in the multiple-entry key*

(N.B. Except where the contrary is indicated, the terminology follows the recommendations of the Committee on Nomenclature (I.A.W.A. 1933) set up by the International Association of Wood Anatomists.)

Growth Rings

Growth rings present.
Ring porous.
Intermediate ring porous.

[1] Blank cards of this type are obtainable from Messrs Copeland and Chatterson, Exchange House, Old Change, London, E.C. 4.

Vessels

Exclusively solitary (pore multiples rarer than about 1 in 50).
Radial multiples of 4 or more common (intended for use where the grouping dominates the vessel pattern, e.g. *Olea*).
Radial or oblique arrangement (e.g. *Quercus*, *Calophyllum*).
Tangential arrangement (e.g. *Ulmus*).
Pore clusters present (e.g. summerwood of *Morus*, *Cordia*).
Perforations exclusively simple.
Multiple perforation plates present.
Perforation plates with more than 20 bars.
Spiral thickenings present.
Intervascular pits minute (not more than about 0·003 mm. diameter, e.g. Meliaceae, *Betula*).
Intervascular pits arranged horizontally and/or scalariform.
Intervascular pits vestured.
Tyloses abundant.
Tyloses sclerosed.
Coloured or white deposits, or gum, present in heartwood.
Fewer than 5 per sq. mm. (individual vessels of multiples all counted).
Fewer than 20 per sq. mm. (individual vessels of multiples all counted).
More than 40 per sq. mm. (individual vessels of multiples all counted).
Mean tangential diameter less than 50 μ.
Mean tangential diameter less than 100 μ.
Mean tangential diameter greater than 200 μ.

Fibres

Septate.
Thick walled (lumen less than one-half thickness of single wall).
Pits distinctly bordered.

Tracheids

Present.

Parenchyma[1]

Predominantly apotracheal.
Diffuse.
Predominantly paratracheal.
Vasicentric.
Aliform, or with a few confluent bands.
Banded.
Bands uniseriate.
Bands usually not less than 4 cells wide.
Bands usually not less than 6 per mm.
Storied.
Fusiform parenchyma common.

Rays

Commonly exceeding 1 mm. in height.
Exclusively uniseriate (occasional biseriations disregarded).
Commonly more than 4, but less than 11, cells in width.
Commonly exceeding 10 cells in width ("broad rays").
Aggregate rays present.
Of two distinct widths (uniseriate and exceeding 4 cells wide).
Homogeneous[2] (i.e. marginal cells less than twice height of ordinary procumbent cells, as seen in radial sections).

[1] The treatment of parenchyma is that of Chalk (1937); the term apotracheal includes diffuse and metatracheal as defined in the I.A.W.A. glossary.

[2] For the purpose of the key it was necessary to extend the I.A.W.A. definitions of homogeneous and heterogeneous by introducing an arbitrary standard of marginal cell shape.

Marginal rows 4 or more cells high.
Marginal rows frequently exceeding 10 cells high.
Bi- or triseriate portions no wider than uniseriate portions (e.g. many Rubiaceae).
Tile cells present.
Sheath cells present.
Intercellular canals, or latex tubes, present.
Storied.
Commonly less than 4 per mm. in transverse sections.
Commonly more than 12 per mm. in transverse sections.
Pits to vessels large in comparison with intervascular pits.

Other Features

Included phloem present.
Vertical intercellular canals present.
Vertical intercellular canals in tangential lines (excluding obviously traumatic canals).
Crystals in ordinary cells.
Crystals in chambered cells.
Crystals in idioblasts.
Raphides or druses present.
Oil or mucilage cells present.

Physical Properties

Distinct odour.
Heartwood distinctively coloured.
Match-size splinter burns to complete ash (only recorded where likely to be required in separation).
Specific gravity (air-dry) less than 0·4.
Specific gravity (air-dry) greater than 1·0.

Geographic Regions

(1) Europe, Africa north of Tropic of Cancer, Central and Northern Asia, including Arabia, Baluchistan, Afghanistan, China, Japan and Formosa.
(2) India, Ceylon, Burma, and Southern Tibet, Andaman, Nicobar, Laccadive and Maldive Islands.
(3) Malay Peninsula, Indo-China, Malayan Islands, Philippine Islands, New Guinea and other islands of Polynesia.
(4) Australia and New Zealand.
(5) Tropical Africa (between Tropics of Cancer and Capricorn). Madagascar, Mauritius, Bourbon, Seychelles, and smaller neighbouring islands.
(6) Africa south of Tropic of Capricorn, Tristan da Cunha, Gough, St Paul, and Amsterdam Islands.
(7) North America (Canada, United States, Greenland, Bermudas).
(8) Central America (including Lower California), West Indian Islands, Tropical South America (including Brazil, Paraguay, Bolivia, and Peru).
(9) Temperate South America (including Chile, Argentine, Uruguay), Juan Fernandez, Falkland Islands, and South Georgia.

ACKNOWLEDGEMENTS

In so far as the key is applied to wood, the principal feature is a classified list of characters which have been found useful in the identification of hardwoods. A preliminary list, prepared by the writer, was circulated to a number of laboratories early in 1937, and in response suggestions were received from interested workers, several of whom wished to adopt the method in their own laboratories. These suggestions have been embodied as far as possible in the present list, together with amendments made as the result of experience in the use of the key in this Laboratory. In collating the suggestions of other workers the writer has had the assistance of Dr L. Chalk who recently visited a number of laboratories abroad and discussed the preliminary list of characters with the workers concerned. It is desired to acknowledge the co-operation of the following, several of whom have given much time and thought to the work: Dr A. T. J. Bianchi, Proefstation v.h. Boschwezen, Buitenzorg; Mr C. E. Carter, Australian Forestry School, Canberra; Dr L. Chalk, Imperial Forestry Institute, Oxford; Dr M. M. Chattaway, Imperial Forestry Institute, Oxford; Mr K. A. Chowdbury, Forest Research Institute, Dehra Dun; Mr H. E. Dadswell, Division of Forest Products, Melbourne, Australia; Mr H. E. Desch, Forest Research Institute, Kepong, F.M.S.; Prof. K. S. Harrar, Duke University, Durham, North Carolina; Mr A. Koehler, Forest Products Laboratory, Madison; Mr C. J. J. Watson, Queensland Forest Service; and Mr M. B. Welch, Forestry Commission, New South Wales.

The writer is particularly indebted to his colleagues, Mr B. J. Rendle, in charge of the Section of Wood Structure, and Mr E. W. J. Phillips, for a close interest and many valuable suggestions throughout the development of the key; and to Mr W. A. Robertson, Director of Forest Products Research, for permission to publish this account.

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NOTES

ON THE SUPPOSED ACCUMULATION OF FAT IN CONIFER LEAVES IN WINTER

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REFERENCE is still frequently made in current literature to the supposed replacement in winter of carbohydrate by fat which is claimed to accumulate in the leaves of evergreen trees and shrubs during that season. This idea seems to be based principally on the authority of papers by Tuttle (1919, 1921), Lewis & Tuttle (1920, 1923) and Korstian (1924). As an example, Warden (1935), recording a better development of the endodermis in plants of *Senecio vulgaris* growing in winter, suggests that under cold conditions fat may be produced at the expense of carbohydrate and continues that "it should be noted that several investigators have shown that many plants partly or wholly replace carbohydrate by fatty material during the winter months". Reference is given to the 1923 paper of Lewis & Tuttle. Although the inadequacy of the data on which this idea is based was shown many years ago (Doyle & Clinch, 1927), the repeated recent references seem to render it advisable to call attention again to the actual facts of supposed fat accumulation as far at least as they relate to the leaves of conifers.

Examination of the pertinent papers shows that they present no evidence either of the presence of any fat at all or of any seasonal change in fat. The so-called fat was demonstrated microchemically with neutral red and osmic acid. Nothing is said of the results of the neutral red tests, reliance being placed on the blackening obtained by treatment by osmic acid. It is not easy to determine from the papers what is the distribution in the cells of the material so blackened, but in some cases, *Picea* for instance, it is densely and evenly present in the vacuoles. No data whatsoever, qualitative or quantitative, are given to show any seasonal difference in this substance reacting with osmic acid. The papers, it may be added, deal with many aspects of the conifer leaf in winter and may only refer to fat incidentally. Thus in the paper (Lewis & Tuttle, 1923) actually referred to by Warden (1935) the only reference to fat is literally confined to the single statement that in January in the mesophyll cells of *Picea*

"the green colour was confined to a small portion of the cell *while the major portion was occupied by fat*". In short these papers merely state the presence in the leaves in winter of a substance which gives a black reaction with osmic acid.

These results have already been commented on by Doyle & Clinch (1927) as far as they refer to conifer leaves. Undoubtedly there are frequently present in the cell vacuoles of these leaves considerable quantities of a substance blackening with osmic acid. They point out however the notoriously obvious fact that osmic acid is not a specific fat test; that most strongly reducing substances give a similar reaction; that conifer leaves are very rich in such substances; and that the material does not even react with Sudan III or give other normal fat reactions. Some fat reactions are given, however, by small droplets sparingly present in the cytoplasm at all seasons. These had already been investigated in *Taxus* by Meyer (1918), even before the first of the Tuttle articles appeared, and, from a detailed microchemical study, he concluded that these droplets are not fat (they are probably some resiniferous secretion) and show no seasonal change except a slight increase in size with age. Doyle & Clinch were also unable to demonstrate lipase in the leaves at any season of the year.

Thus there has been presented so far no evidence that fat accumulates in conifer leaves in winter or that fat at any season plays any conspicuous part in their metabolism.

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AN AUTOMATIC APPARATUS FOR THE DETERMINATION OF VOLUMES OF SMALL BLOCKS OF WOOD OR SIMILAR OBJECTS

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(With 1 figure in the text)

THE apparatus was designed to determine the volumes of small blocks of wood, accurately, without wetting them, by displacement of mercury. The general layout of the apparatus can be seen in the sketch. The supporting framework is a robust construction of wood.

The main feature of the apparatus is the automatic electric control, which adjusts the mercury to a predetermined level. It consists of two steel knitting-needles *A* and *B*, bound tightly together, but insulated from each other by small pieces of rubber or cork. The needles are arranged so that the point of one, *B*, projects about $\frac{1}{4}$ in. beyond the point of the other. The top 2 or 3 in. of *A* is bent away from *B*, and to the top of *B* is fixed a small bolt so that the needles can be quickly and firmly fixed at a constant level by clamping the shank of the bolt, pushed as far as the head will allow in the hole of the wireless terminal *T*. The terminal is fixed to a metal bridge *H*, which is in turn fixed to the wooden support by bolts. The needles project down the axis of the glass tube *C*, which has a glass tap *D* fused to the bottom by which the mercury is run out. The tap is turned by rotating a coaxial spindle *E*, on bearings *LL*, a claw holding the handle of the tap. The spindle *E* is fixed by means of a coupling *G* to a long arm *F*, which bears at its extremity a soft iron plate. The arm is weighted so that the electro-magnet *MM* will hold up the arm, but on release the arm falls rapidly, turning the tap *D*. The extra tube *R* with its glass lead and tap is used to fill *C* without including air bubbles.

The electric connexions are made from one side of a low tension direct current supply (2, 4, or 6 volts according to the size of the magnet) through the magnet coils to one of the bolts holding the bridge *H*. This enables connexion to be made to the needle *B*. When the tube *C* is filled with mercury above the level of the point of the needle *A* the circuit is completed back to the low tension supply via a wire from the top of *A*.

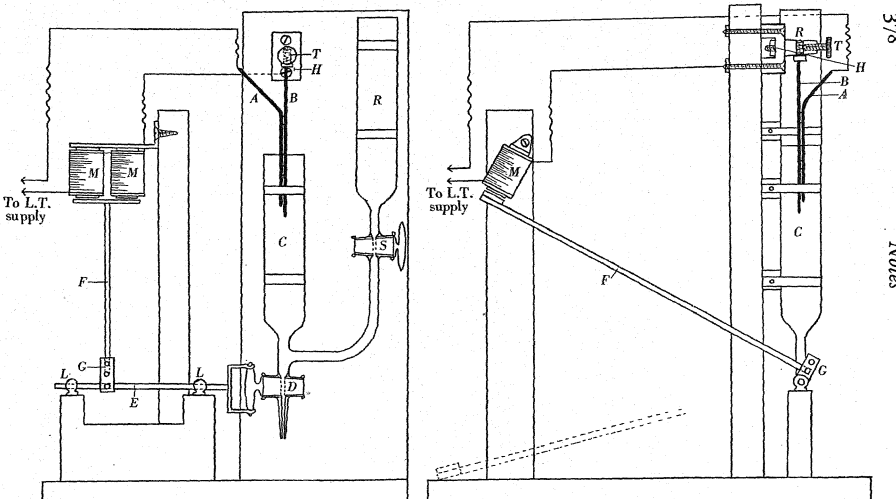
To L.T. supply
M
M
T
H
A
B
R
C
S
F
G
L
L
E
D
Front elevation
To L.T. supply
M
R
T
H
B
A
C
F
G
Side elevation

Fig. 1.

The method of working is as follows. Mercury is run into *C* from *R* by opening the tap *S*, which is closed when the level of the mercury is above the point of needle *A*. The circuit is now completed and on lifting up the arm *F* to the magnet, the soft iron plate is held there. The rate of flow of mercury from *C* is adjusted by loosening the coupling *G*, and rotating the spindle *E* until a drop by drop flow is obtained. The coupling is then tightened. The mercury continues to run out until the level falls just below the point of *A*, when the circuit is broken and the arm *F* falls, turning the tap *D* and stopping the flow. The needles are taken out by unscrewing the terminal *T*, the block is put in and pushed under by the point of *B*, the needles being again fixed in position. The displaced mercury rises and completes the circuit. On raising the arm *F* the magnet holds the soft iron plate and mercury runs out from *C*, until the circuit is broken on reaching the same level as before. The volume of the displaced mercury can be found by weighing.

The apparatus is most accurate when the tube *C* is only slightly larger in bore than the block, and when the rate of flow is very small.

The following Meccano parts have been found very satisfactory:

Spindle *E*-axle rod, No. 15. Arm *F*-axle rod No. 13.

Bearings *LL* (2), part 136. Coupling *G*, part 63.

The bridge *H* can be made from strip metal or block as desired. The electro-magnet is made from bell coils.

Using metal cylinders of known volume, between 4 and 10 c.c., the mean error has been found to be about 0·5% on single readings. With wood blocks, there may be penetration of mercury, but this is usually very small or can be corrected for. With a set of 16 blocks of elm the mean penetration was 0·033 c.c., the maximum being 0·070 c.c., the minimum 0·0061 c.c. As the volumes of the blocks was about 12·5 c.c., the error is negligible. With a set of 14 oak blocks with a mean volume of 11·0 c.c., the mean penetration was 0·007 c.c. The correction is made, if necessary, by weighing the blocks before and after immersion, and adding the increase in weight to the weight of the displaced mercury.

REVIEWS

The Origin of Life. By A. I. OPARIN. Translated by SERGIUS MORGULIS. $8\frac{1}{2} \times 5\frac{1}{2}$ in. Pp. 270+viii with 8 figs. Macmillan, New York, 1938. Price 8*s.* 6*d.*

Prof. Oparin disarmingly calls his book a weak attempt to picture the evolution of life without losing contact with the ground of scientifically established facts. The subject is carried in turn through the territories of history, astronomy and geology; and breaks by way of organic and biochemistry into plant metabolism. Under the first heading there is a straightforward review, starting with the dead hand of Aristotelian entelechy and finishing with the sterility of Pasteur. The conclusion reached is the now fashionable one that life is neither coeval with matter nor liable to generate with sudden spontaneity: nevertheless what cannot be achieved at a jump may yet be evolved; indeed, given geological time and remoteness, nothing seems impossible.

The argument begins with the hypothetical cosmic fission which budded off our planets from the sun. The evolution of organic matter began at the same moment, due to the setting in of cooling and the consequent precipitation of carbon. The characteristic of the author's hypothesis also enters at this early stage and lays the restrictive basis with which evolution has, he supposes, ever afterwards had to wrestle. Carbon could only exist in a world cooling through the centigrade thousands in such reduced forms as dicarbon (C_2), methene (CH), methane (CH_4) and cyanogen (CN); and oxidized carbon (CO or CO_2) was impossible. An impressive array of the observations of astrophysics is given here in evidence. The smaller planets retained none of their lighter elements and Mercury (like the Moon) is revealed as a bare rock without an atmosphere at all. Jupiter is "an island of heavy hydrocarbons and ammonia floating on an enormous hydrocarbon ocean"; and its atmosphere consists mainly of methane. Only on the earth and Venus is carbon dioxide demonstrable and free oxygen is equally scarce (the earth and Mars), but water vapour more abundant. Both carbon dioxide and free oxygen are believed to be of secondary and biological origin.

The first evolution of organic matter is therefore believed to have been anaerobic and is attributed to the interaction of the still hot carbides and hydrocarbons with superheated water vapour. Evidence is drawn from modern high temperature experiments on mineral oils. Hydration of the carbon occurs readily with the formation of such compounds as acetaldehyde; and the presence of reduced nitrogen leads to the synthesis of aldehyde-ammonias. The further evolution of such simple substances—homogeneously distributed in a cooling ocean—into organized matter has two aspects, the development of the necessary catalytic systems and the emergence of colloidal discontinuity. On both points the author has something to say.

The final chapters deal with the evolution of metabolism. In line with the foregoing, anaerobic processes are regarded as primitive and aerobic processes as evolutionary advances only possible when the earlier biological activities have provided the necessary reagents. Thus aerobic respiration develops out of fermentations, and carbon dioxide assimilation is a successor to "saprophytic" assimilations whose initial unorganized organic matter arose from the reactions already mentioned. A most attractive feature of this hypothesis is, of course, its explanation of the immense importance of water as a reactant in biological oxidations.

It is inevitable in such a book that there should be general statements that it might prove embarrassing to have to establish in detail (e.g. "the basic respiratory mechanisms so well defined in the higher plants..."). As entertaining and suggestive biological speculation it is sure to have many appreciative readers.

W. O. JAMES.

Marine Algae of the North Eastern Coast of North America. By WILLIAM RANDOLPH TAYLOR. University of Michigan Press. 427 pp. and 60 plates. Price 5 dollars.

The book comprises a short introduction touching briefly on the character and extent of the area surveyed; a systematic list; a descriptive catalogue of 393 species belonging to 165 genera; a full bibliography and 60 beautiful illustrative plates.

The publication of this book by one who has himself made notable contributions to the content of knowledge in algology is very welcome. The book is a descriptive catalogue of the marine algae distributed on the north-east coast of America. Locality for each species is stated but no other ecological data are given. The systematic list offers a convenient method of grouping but makes no claim to represent a natural system of classification. The question of relationships is not discussed nor is the book intended to serve as a text-book of marine algae. It is, however, clearly designed to meet the needs of the general student of Botany. The Introduction contains much good advice, clearly the result of long experience, on the collection and preservation of marine algae; the rest of the book offers the student an opportunity of identifying his specimens.

For this purpose, the book is provided with artificial keys leading successively to large groups of algae, to families and finally to genera, species and varieties. The criteria on which these keys should be constructed have presented a difficulty which has been frankly admitted by the author. In making the key leading to the larger groups, the author has made use of facts of life-cycle or details of development of reproductive organs which are unlikely to be determined by inspection of the specimen the identification of which is desired. For example, with an unknown member of the Rhodophyceae, a knowledge of the form of the carpogonium and the behaviour of the auxiliary cells is necessary before any further step towards identification can be taken. Similarly a knowledge of the details of gametophytic as well as sporophytic phase in the life cycle of a member of the Phaeophyceae is demanded. The author maintains, however, that the student will readily learn by experience how to place his specimens in the right group, and, for his further exploration of genera and species, provides a series of keys based on broad and readily observable morphological features. These keys are well constructed and easy to use.

Descriptions of species are concise but sufficiently detailed to give the student an adequate picture of the plant in question and to enable him to test the accuracy of his use of the artificial keys. In compiling the descriptions the author states that he has not adhered strictly to the original description given by the founder of the species. He has drawn largely on his own intimate knowledge of the plants and has supplemented it from the writings of others who have made special studies of individual species or genera. He warns the reader that any description is to be regarded as the author's own concept of any given species.

All algologists will sympathize with the author on this point. Original descriptions of algae written more than relatively few years ago are apt to preserve an impenetrable silence on features now realized to be of critical importance and to be framed in terms the significance of which has long disappeared.

The plan of the book is very simple and its form is attractive. Advanced students will find that it serves their needs adequately, especially in the lists of relevant literature subjoined to each description. All readers will agree in admiring the beauty of the illustrative plates. The medium chosen is line and stipple done on excellent paper. The drawings reveal that gift, unfortunately all too rare, by which real artistry and scientific accuracy are combined to present a series of convincing pictures in which general form and minute histological detail are equally well portrayed.

M. KNIGHT.

Galapagos Observado Fitologicamente. By M. ACOSTA SOLIS. Pp. 78, 30 photographs. Imprenta de la Universidad Central, Quito, Ecuador. 1937.

An account of the geography, geology and climate of these interesting islands is followed by an account of the vegetation, based on the observations of the author during a short visit, and those of other biologists from Darwin to the present time. There are two well-marked types of vegetation, xerophytic in the lower parts of the islands and mesophytic in the higher parts, but there is no sharp line between the two as they are determined by the rainfall, which increases with the altitude. In the lower region the growth of the perennial species is slow, being restricted to the short rainy season, and they appear to attain a great age. The majority of species flower towards the end of this season. In the higher parts growth is continuous and there does not seem to be any well-defined flowering period, though, as the author points out, a much longer visit is necessary to settle this and other points definitely. The plants of the coastal "Opuntiales" often have the green colour of their assimilatory tissue masked by other pigments, and it is suggested that this may be due to the bright sunlight to which they are exposed. An investigation of the morphology of the photosynthetic regions of these plants is being made. The majority of plants are wind pollinated, examples of bird pollination being very few in spite of the abundance of small birds and the relative frequency of this method of pollination amongst the plants of the mainland. All the usual methods of seed dispersal are found, and a list of the types of mechanism found in the different families is given. The booklet concludes with a list of the flora and an account of the agriculture of the islands. The work suffers somewhat from the almost complete absence of good libraries and good printers in South America. Errors have crept into many of the plant names, the method of citation of authorities is erratic, all the liverworts are kept in the "genus" *Jungermania*, and the photographs are badly reproduced.

T. G. TUTIN.

SOCIETY FOR EXPERIMENTAL BIOLOGY

The Society for Experimental Biology holds three conferences each year. Short reports of proceedings of botanical interest appear in this *Journal*. Members of the Society can take the *New Phytologist* at special rates. Further particulars can be obtained from the botanical Secretary, Prof. T. A. Bennet-Clark, University College, Nottingham.

FORTY-SECOND CONFERENCE

The forty-second conference was held in March at the John Innes Horticultural Institution. Among the meetings of special interest to botanical members was a discussion on the gene concept opened by Prof. J. B. S. Haldane and contributed to by many speakers.

In a session of papers on genetical problems (Prof. Haldane in the Chair), Dr Price reviewed the chemistry of anthocyanin pigments, pointing out that cyanidin is both synthetically simpler than pelargonidin and delphinidin, and that the latter two predominate in the flowers of the more advanced families. Coloured leaves and fruits in most cases contain derivatives of the relatively simple cyanidin. Dr Lawrence, dealing with genetical aspects of pigmentation, pointed out that recent work has shown that genes controlling colour are highly specific in chemical action. Genes have been found which control oxidation and reduction, methylation, conversion into glycoside, etc., of the anthocyanidin molecule. Genetic evidence of a common origin of flavone and anthocyanidin pigments exists. Successive stages of the synthesis are controlled by different genes.

Dr Lawrence took the Chair at a session of papers on Cell Mechanics at which Drs Darlington, Thomas and Klingstedt described evidence bearing on the relationships of the chromosome with the spindle at meiosis. The separation of the paired chromosomes depended on the centromeres being held close together by chiasmata between the chromosomes. Their movements were shown to be related to movements of centromeres in the prophase nucleus before the spindle was formed. On the other hand, division of univalents depended on the orientation of their individual centromeres. These movements are held to depend on a change in the centromeres preparatory to division. All these movements, it was suggested, are conditioned by the fibrous structure of the spindle and may have an electrostatic basis. The mechanism by which electrostatic forces might bring about such movements aroused some discussion to which various members contributed.

There were many demonstrations of cytological and genetic material and also demonstrations of micro-methods for recognition of anthocyanin pigments.

FORTY-THIRD CONFERENCE

This conference was held in Aberdeen in July 1938. Two sessions of botanical papers were held at which Profs. Matthews and Steven took the Chair. Members were also entertained at and saw over the Macaulay Institute for Soil Research.

Dr Gross described the osmotic system of the plankton diatom *Ditylum brightwelli*, the protoplast of which is found to shrink as in plasmolysis when cells are transferred from sea water to unbalanced saline or sugar solutions either isotonic with or hypotonic to sea water. Recovery takes place on return to sea water. "Plasmolysis" is also brought about after several hours by darkening the cells. These results suggested that the internal osmotic pressure

of the turgid cell is lower than that of sea water, a condition which entails continuous work against the outside osmotic pressure.

Dr Clouston discussed the rolling mechanisms of grass leaves and expressed the view that in the more xerophytic types the hinge cells play a rather subordinate part as compared with turgor changes in the mesophyll. On the other hand, *Arundo*, *Glyceria*, and many others have well-developed motor cells the turgor changes of which fold the leaves.

Dr Meiklejohn gave an account of a new type of denitrifying bacteria from soil which reduce nitrate with production of gaseous nitrogen under aerobic conditions. They are active within the *p*H range 5·5–9·5. Nitrite is formed as an intermediate product, but it is toxic except in very low concentrations. The loss of nitrogen as gas from cultures may be as much as 80 % in 7 days.

Dr Stewart described the soil fertility investigations in progress at the Macaulay Institute in which an attempt is being made to correlate pot and laboratory experiments with field data. Most criticism centres round the question of the constancy of Mitscherlich's Wirkungsfactor.

Dr Warne described work on rootstock-scion relationships in Apple. His data indicated that early cessation of growth in such stocks as M 9, which are associated with precocious fruiting, does not appear to be due to deficiency of supply of mineral elements or nitrogen or to any internal deficiency of water. It is not possible to put forward an explanation of the rootstock effect at present.

Mr W. G. Campbell described some of the work carried out at Princes Risborough on chemical changes effected by wood-destroying fungi and by *Lyctus* powder-post and death-watch beetles. Mr Boswell dealt with the biochemistry of attack by *Merulius lachrymans*, and pointed out that the lignin and benzene-soluble fractions were unaffected by the fungus. From the nature of certain degradation products isolated it was concluded that the preliminary stages of cellulose breakdown are hydrolytic, yielding molecules consisting of not less than three glucose residues, and that these latter are oxidatively attacked by the fungus.

Dr T. E. T. Bond described work on infection of tomato and related plants by *Cladosporium fulvum*. Stomatal penetration occurs both on tomato and on plants which are immune. Experiments suggest that hydrotropic stimuli affect penetration. A paper on soil Mucorales by Dr M. Campbell was given *in absentia*.

The forty-fourth conference will be held at the Royal Veterinary College, London, N.W. 1 on 19, 20 and 21 December 1938.

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AN EXPERIMENTAL INVESTIGATION OF THE MECHANISM OF STOMATAL MOVEMENT, WITH SOME PRELIMINARY OBSERVATIONS UPON THE RESPONSE OF THE GUARD CELLS TO "SHOCK"

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(With Plate V)

INTRODUCTION

SINCE the experiments of von Mohl (1856) and the observations of Schwendener (1881) the motility of the stomata has generally been attributed to changes in turgor of the guard cells. In support of this view, much evidence has accumulated from observations of the changes in osmotic pressure of their cell sap relative to that of the other epidermal cells (Iljin, 1914; Wiggans, 1921; Steinberger, 1922; Sayre, 1926; Strugger & Weber, 1926). The change in the amount of starch in the guard cells when the stomata open or close (Darwin, 1898; Lloyd, 1908; Iljin, 1914; Sayre, 1926), and the inverse change in their sugar content (Sayre, 1926), provide an explanation of the observed variation in osmotic pressure. These carbohydrate transformations have generally been assumed to be enzymatic reactions, probably due to diastase (Hagen, 1918; Loftfield, 1921; Sayre, 1926). Sayre (1926) suggests that they are governed by changes in pH of the sap of the guard cells. Such hydrolysis of starch or its condensation would appear to be too slow however to account for the rapidity with which stomata open and close. Thus Gregory & Pearse (1934, 1937) find that in *Pelargonium* opening of the stomata can be detected within 1 min. of illumination by means of the resistance porometer. Scarth (1926, 1927*a*) has advanced the

theory that the immediate opening of stomata in the light is brought about by an increase in the pH of the sap of the guard cells causing the imbibitional hydration of amphoteric colloids which would provide a rapid increase in turgor. In the dark, when the pH falls owing to the accumulation of CO_2, the colloids approach their isoelectric point and the bound water is released and escapes to neighbouring epidermal cells. Scarth (1927*a*) says: "Of course only a part of the change in turgor is ascribed to the above cause, but it is that part which is responsible for the rapid initial response which is often displayed when light acts upon the cells. Hydrolysis of carbohydrate is a slower but probably more powerful reinforcement." Kisselew (1925) and Nicolic (1925) suggest that changes in the permeability of the guard cells are partly responsible for their changes in turgor, and Kelle (cited by Bünning, 1936) has noted an increase in permeability when the stomata are open in the light.

Apart from the turgor mechanisms discussed above, there is another possibility, namely that the movement of the guard cells is due to a cell wall mechanism. This might be achieved by changes in the relative imbibitional swelling of two or more lamellae making up the guard cell wall. Thus Nadel (1935) finds that the stomata of citrus and potato which have been killed by "fixatives" can be made to open and close by transference through various liquids. On the basis of the appearance of the stomata when examined by polarized light she concludes that these movements are brought about by differences in swelling of the walls in the different liquids. She cites a view of Pringsheim that possibly even in life swelling mechanisms play an important part in stomatal movements. Nadel attributes the observed movements of the dead stomata to the outer walls of the guard cells being composed of two layers formed by cutin and cellulose respectively. In this connexion it may be pointed out that the mechanism would function even if both layers were cellulose, as long as the micellae were differently orientated in the two layers, since swelling would be greatest in the directions at right angles to the length of the micellae. Nadel suggests that it is possible that these physical movements play a certain role in the reaction of stomata *in vivo*.

It is worthy of note that changes in pH such as found by Scarth (1926, 1932) would presumably affect the degree of hydration of the cell walls and might thus operate a mechanism such as that postulated by Nadel, or one in which both layers were of the same material with differently orientated micellae.

A decisive experiment to test whether a cell wall mechanism plays any important part in the movements of living stomata was suggested to the author by Prof. F. G. Gregory. If the mechanism is purely one of turgor, on suddenly releasing the sap from the guard cell of an open stoma the cell should collapse at once and diminish the pore to half the aperture. If, on the other hand, a cell wall mechanism is concerned, the stoma should either open wider or at least remain stationary for an appreciable time.

Considering the more frequent type of stoma in which opening is attended by lateral deformation of the subsidiary cells, the pressure exerted must be due either to turgor changes, as is generally held, or to a pressure applied to the guard cell contents by the wall adjacent to the pore (ventral wall) and transmitted to the neighbouring cells. A less probable alternative would be attraction exerted by the dorsal wall of the guard cell, but anatomical considerations discount such a view. Both turgor changes and wall effects may be operative. In either case puncturing the subsidiary cell should lead to stomatal opening, but puncturing the guard cell would appear to offer a means of discrimination, for with a turgor mechanism alone at work, the pore should immediately decrease to half the aperture, while a wall mechanism would lead to an opening if the ventral wall were concerned.

In the case of the less common "bellows" type of stoma, where the guard cells expand at right angles to the epidermal plane, it would appear that on a wall mechanism hypothesis, if no change in volume of the guard cells normally occurs with opening, the aperture should be unaffected by release of the sap. If, on the other hand, an increase in volume occurs with normal opening, then in the open stoma a wall mechanism would presuppose a tension and the pore should enlarge on release of the cell sap.

If puncturing the subsidiary cell of an open stoma is found to lead to further opening, a pressure on the wall of the guard cell remote from the pore is demonstrated. In such a stoma, as stated above, a ventral wall mechanism if contributing to the pressure should bring about an opening when the guard cell is punctured. It is conceivable, however, that the main opening force might be provided by increase of turgor, but that a ventral wall movement might play a small part, especially in the early stages of opening. In the wide open stoma the ventral wall might then be under tension and puncturing the guard cell might lead to partial closure. Such closure should stop short before the stomatal aperture was reduced to one-half.

EXPERIMENTAL

The experiments described below were made with a simple micro-manipulator designed and constructed by Mr E. E. Pyke of this Department. It was found necessary to use a stout glass needle terminating in a short but sharp point in order to penetrate the cell walls. Such needles are conveniently made in the following manner. In a fine capillary tube about 1 mm. diameter a very thin neck is drawn out in two stages after heating in a small flame. The neck is then severed by a red hot platinum wire, in an adapted dry battery gas-lighter, which is brought into contact with the finest part of the neck while the capillary tube is under tension. The microscope used was a Beck Massive Model, with a Wenham type binocular which was found to be very advantageous for micro-dissection on account of the good stereoscopic vision it affords. The micro-manipulation was carried out under a Beck 16 mm. apochromatic objective with Beck 25× compensating eyepieces. The source of illumination was a low-voltage lamp (12 W. for the observations and 24 W. for the photographs) with bull's-eye and water screen, the leaf being illuminated from below by a condenser. The photographs (except Pl. V, fig. 6) were taken using a Beck 4 mm. apochromatic objective, corrected for the uncovered objects by the correction collar, and a Leitz 10× Periplanatic eyepiece in a Leica attachment. Pl. V, fig. 6 was taken with the 16 mm. objective and a 25× compensating eyepiece.

The work was carried out during November and December using *Tradescantia zebrina* and *Cyclamen persicum*. The shoots (*Tradescantia*), or petioles (*Cyclamen*) of the leaves used were cut under water and attached to a glass tube full of water by the method described by Gregory (1938). The leaves or shoots were suspended upside down by the glass tubes under a 1000 W. lamp with a cooling screen of running water. In the case of *Tradescantia* it was found necessary to set up the shoots on the day before the experiment as practically all the stomata closed by about 11 a.m. even under the 1000 W. lamp. The *Cyclamen* leaves, on the other hand, could be set up on the day of experiment as the stomata continued opening throughout the day. These results both for *Tradescantia* and *Cyclamen* are in agreement with the changes of osmotic pressure in the guard cells found by Wiggans (1921) at about the same time of year. For an experiment the glass tube was held in a retort clamp so that the leaf was supported with the lower surface uppermost on the stage of the microscope.

(i) *Cyclamen persicum*

In *Cyclamen* the epidermal cells surrounding a stoma are very irregularly arranged. For the sake of convenience, however, a cell adjacent to the side of a guard cell (see Pl. V, fig. 5) will be termed a "subsidiary cell". In twenty-three cases one subsidiary cell was punctured, six of the stomata being closed and seventeen being open to various extents. When a subsidiary cell of an open stoma was punctured, there was always some degree of enlargement of the adjacent side of the pore, sometimes very slight, sometimes considerable. Such enlargement was usually completed in about 1½ min. An average case is illustrated in Pl. V, figs. 1 and 2. In some cases the adjacent guard cell could be seen to bulge outwards. No subsequent "shock" closure was observed even after 10 min. Of the six closed stomata, three opened to some extent, the other three being unaffected. These results demonstrate a pressure on the dorsal wall of the guard cell in all open stomata and even in some closed ones. The existence of this pressure is also indicated by the initial stomatal opening on wilting found for *Cyclamen* by Gregory & Pearse (1934).

In two cases the epidermal cell at the end of an open stoma was punctured. As might be expected, there was no further opening, nor was any closure observed either immediately or during the period of observation (6 min.).

Fifty-two experiments were performed in which one guard cell of an open stoma was punctured. In none of these was any opening movement observed, and in all cases the punctured guard cell collapsed and reduced the pore to about half the aperture (see Pl. V, figs. 3 and 4). In thirty-five of the experiments such closure was almost instantaneous on withdrawal of the needle, and the final straightening of the ventral wall of the affected guard cell usually occurred within 2–10 sec. In eleven cases the closure was noted as less rapid, approximately 20–30 sec., and five closed very slowly. (One experiment was doubtful, see below.) For reasons given in the Introduction above, it was important to determine whether on puncturing the guard cell of a wide open stoma the immediate closure stopped short before the aperture was reduced to half. In the great majority of cases there was no evidence of this, closure proceeding uninterrupted, though of course with some slowing up in the final stages, until the ventral wall appeared as a straight line. In a few cases, however, it certainly appeared to occur. These exceptions, as also the less rapid closure noted in sixteen of the fifty-two experi-

ments, might well be explained by coagulated protoplasm clogging the puncture. In this connexion it should be noted that Scarth (1927*b*) finds that if a puncture in a plant cell wall is small enough it is readily clogged by coagulating protoplasm on withdrawal of the needle, and the lost turgor is regained.

In eight of the fifty-two experiments the needle was left in the guard cell for an appreciable time (up to 20 sec.). In seven of these there was no visible effect upon the guard cell until the needle was withdrawn. This also is in agreement with Scarth's findings for other plant cells. The guard cell in the eighth experiment collapsed slowly before the needle was withdrawn. It therefore provided the doubtful case on rate of closure mentioned above.

The unpunctured guard cell appeared quite unaffected in fifty-one of the fifty-two experiments (Pl. V, fig. 4), and even when observed for as long as 20 min. showed no sign of "shock" closure. In the exceptional case, the second guard cell slowly closed. No explanation can be offered, unless the second guard cell was accidentally punctured, which was seen to occur in one other case not included.

A few experiments were carried out to discover whether other stimuli would produce "shock" closure of the stomata in *Cyclamen*. In three experiments the cut end of the petiole of a leaf was burnt while open stomata (two, four, and nine in number respectively) were observed under the microscope. No sign of closure was seen in: (i) 15 min., (ii) 102 min., (iii) 34 min. In experiment (ii) a burn was then made with a hot glass rod 1 cm. from the stomata under observation. Again there was no response in the next 13 min. In experiment (iii) further burns were made with a red hot platinum wire (in an adapted gas-lighter) about 2–3 mm. away, first on the petiole side of the stomata and finally in a continuous ring 4–6 mm. diameter. In another 20 min. one stoma appeared to have closed somewhat, the other eight being unaffected. Two porometer experiments, in which the leaf was pressed fairly tightly between the cup with gelatine washer and a glass plate, failed to show any evidence of "shock" closure such as occurs for example in *Pelargonium*.

(ii) *Tradescantia zebrina*

The stoma of *Tradescantia zebrina* together with the four or sometimes five cells surrounding it forms a "unit" which has obviously been derived from a single epidermal cell (Pl. V, fig. 6). In addition to the two "subsidiary" cells flanking the guard cells there

are two others which will be termed "end cells". Occasionally a wall bisects one of these, running from the end of the stoma to the neighbouring epidermal cell.

In thirty-one experiments, one subsidiary cell was punctured, fourteen of the stomata being closed, five very slightly open, and twelve open. Of the twelve open stomata, eleven showed some degree of enlargement of the pore on puncturing, sometimes accompanied by noticeable bulging outwards of the adjacent guard cell. In all these cases, as well as the one which showed no such opening, a closing movement followed. Thus in six of them the adjacent guard cell moved first to give a straight ventral wall within 1 min. 50 sec., the other guard cell completing the closure within eight minutes. In three cases the adjacent guard cell collapsed but the other guard cell appeared unaffected, in one both guard cells moved simultaneously to complete closure in 3 min., and in two cases the adjacent guard cell was unaffected while the other guard cell collapsed. None of the five experiments with very slightly open stomata produced an enlargement of the aperture and in every case the whole stoma closed in from 30 to 55 sec. The fourteen experiments with closed stomata also failed to show any opening movement. These experiments therefore demonstrate the existence of a pressure on the dorsal wall of the guard cell in open but not in closed or nearly closed stomata.

Four experiments on open stomata were carried out in which an end cell was punctured. Closure of the whole stoma followed in 40 sec. to 4 min.

In fifteen cases, one guard cell of an open stoma was punctured. None of these showed any enlargement of the pore and in thirteen of them the punctured guard cell collapsed instantaneously on withdrawal of the needle, reducing the pore to half the aperture. Of the other two stomata, one showed rapid and one rather slow movement of the ventral wall of the punctured cell. These two exceptions, as in the case of *Cyclamen*, were probably due to the puncture becoming partially clogged by coagulating protoplasm. In all fifteen experiments the other guard cell completed the closure of the pore, the times ranging from 30 sec. to $3\frac{1}{2}$ min.

In one of the above experiments the needle was allowed to remain in the guard cell for 20 sec. before being withdrawn and the latter appeared unaffected during this period.

In view of the "shock" closure which, as described above, invariably followed the puncturing of any one of the cells making up the stomatal "unit", seven further experiments were performed in

which adjoining epidermal cells were punctured. In two experiments the puncturing of a single epidermal cell, adjacent to a subsidiary cell, had no effect upon the open stoma in 15½ and 55 min. respectively. In each case a second cell adjoining an end cell was then punctured. In one experiment there was no closure in a further 33 min., but in the other one guard cell appeared to have collapsed 7 min. after the second puncture. The second guard cell remained unaffected for a further 22 min. In both experiments puncturing a subsidiary cell finally brought about complete closure in 2–3 min. Five experiments were performed in which two epidermal cells, adjoining subsidiary and end cells respectively, were punctured with large holes, one immediately after the other. In every case complete closure of the stoma took place, the times ranging from 1 to 14 min.

Discussion of results and conclusions

In both *Cyclamen* and *Tradescantia* the existence of a pressure upon the dorsal wall of the guard cell of an open stoma has been demonstrated experimentally by puncturing the subsidiary cell. The experiments in which a guard cell is punctured prove that a ventral wall mechanism makes no contribution to this pressure, since no enlargement of the pore occurs. Furthermore, the fact that in nearly all cases the movement of the ventral wall of the punctured guard cell proceeds uninterrupted until it appears as a straight line would seem to show that a wall mechanism plays no appreciable part even in the early stages of opening of the stoma, unless it is confined to what Stålfelt (1927) has called the "stretching phase" ("Spannungsphase"). It is concluded, therefore, that a turgor mechanism alone is concerned in the opening of the stomata of these two species, except perhaps during the initial stage before an aperture appears when the possibility of a wall mechanism exerting an effect is not disproved.

No evidence has been obtained of any response by the guard cells of *Cyclamen* to "shock" or wound stimuli, even the puncturing of one guard cell of a stoma having no apparent effect on the other. In *Tradescantia*, on the other hand, the puncturing of any one of the cells which make up a stomatal "unit" leads to closure. It should be mentioned that such closure might be explained as a purely osmotic phenomenon. The removal of the wall pressure in the punctured cell will allow what sap remains to exert its full osmotic pressure. This will exceed the suction pressure of the adjoining cells, with which

the punctured cell was previously in equilibrium, and hence water will be withdrawn from them and the turgor of the guard cells will fall. The response of *Tradescantia* stomata to the puncturing of other epidermal cells outside the "unit" appears to vary with the extent of the wounding. Further experiments upon this subject and on the effect of other "shock" or wound stimuli will be postponed until the spring when the *Tradescantia* leaves may be expected to be in a more active condition.[1]

Summary

1. Experiments were carried out to determine whether stomatal movement was due entirely to turgor changes, or whether imbibitional changes in a two-layered wall were concerned. The method consisted in puncturing guard cells and subsidiary cells under microscopic observation. The plants used were *Cyclamen persicum* and *Tradescantia zebrina*.

2. In both species movements of guard cells demonstrated hydrostatic pressure on the dorsal wall.

3. In both species the collaboration of a wall mechanism in stomatal opening was definitely disproved, except possibly in the initial phase before any aperture appeared. ("Spannungsphase", Stålfelt).

4. The guard cells of *Cyclamen* appeared to show no response to "shock" or wound stimuli.

5. In the stoma of *Tradescantia*, closure occurred in response to the puncturing of one guard cell or of any one of the four cells which together with the stoma form a unit.

Acknowledgements

The author wishes to thank Prof. F. G. Gregory, who suggested the crucial experiment, for his continued interest and most stimulating discussions. Also Mr E. E. Pyke for the loan of the micromanipulator. The work was carried out while the author was holding a Leverhulme Research Fellowship.

[1] The potted plants used did show some new growth and negative geotropic response.

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Fig. 1

Fig. 2

Fig. 3

Fig. 4

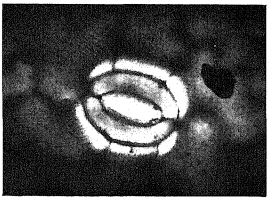

Fig. 5

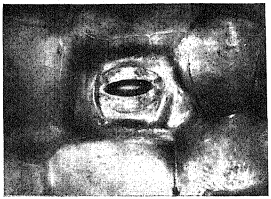

Fig. 6

EXPLANATION OF PLATE V

Fig. 1. Partially open stoma of *Cyclamen persicum*. × 330.

Fig. 2. The same stoma showing further opening on upper side following puncture of subsidiary cell on that side. × 330.

Fig. 3. Open stoma of *Cyclamen persicum*. × 330.

Fig. 4. The same stoma showing reduction of aperture to one-half and straight ventral wall of the guard cell which has been punctured. Photograph taken 10 min. after puncturing, but no movement of uninjured guard cell has occurred. × 330.

Fig. 5. Open stoma of *Cyclamen persicum* fixed by Lloyd's method in a stripped epidermis, showing irregular arrangement of neighbouring cells. × 330.

Fig. 6. Partially open stoma of *Tradescantia zebrina* showing arrangement of cells in the stomatal apparatus within the contour of an epidermal cell. × 230.

MORPHOLOGY OF ABNORMAL FLOWERS IN SOME ANGIOSPERMS

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With 5 figures in the text

In the following study, a description is given of five cases of abnormalities of flowers. The abnormalities fall into two categories, viz. virescence and phyllody of the floral organs and petalody of the stamens. The two categories will be treated separately.

Virescence and phyllody

Tropaeolum majus (garden nasturtium). The phenomena of virescence and phyllody of the floral parts proceed regularly through a series of stages and culminate in the formation of an axial foliar shoot from the flower. The different stages are as follows:

(i) The first sign of the abnormality of the flower is the shortening of the spur, which disappears completely by the time the petals are fully virescent, and the ovary becomes raised on a fleshy stalk, the "gynophore". The carpels are inflated and contain abnormal ovules (Fig. 1*a*). Microtomed and stained serial sections of the ovary were examined for determining the nature of the abnormalities of the ovules. In these, the integuments are greatly enlarged as foliaceous structures, possessing all the characteristic features of the ordinary leaves, such as the stomata in the epidermis, a pigmented mesophyll with the palisade (on the inner surface of the integument) and the spongy tissues and well-developed vascular strands. These vascular strands are separated from a large tracheal mass at the chalazal region of the ovule, from which a strand passes also to the nucellus (Fig. 1*b*). The nucellus, the apex of which is tapering into the micropyle, is small relatively to the size of the integuments. The embryo sac appears as a much reduced cavity, the traces of which may be

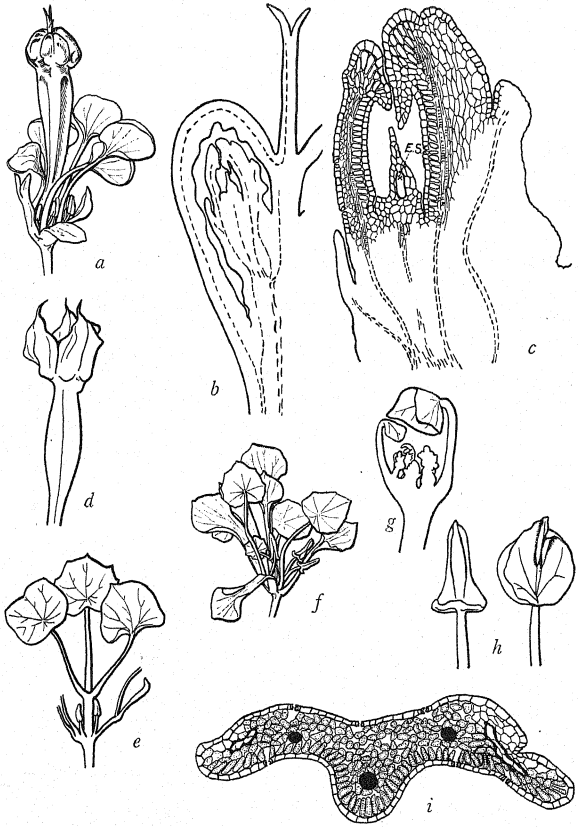

Fig. 1. *Tropaeolum majus.* *a*, *d*, *e* and *f*, different stages in the transformation of the floral parts in the abnormal flowers; *b*, longitudinal section of ovary in *a*; *c*, same showing only the ovary, *E.S.* embryo sac; *g*, the foliar shoot in place of ovary in the flower in *f*; *h*, two stages in the phyllody of the anthers in the same flower; *i*, section of a foliaceous anther.

entirely lost a little later. It may be inferred from these observations, that the phenomenon of phyllody extends to the ovules at quite an early stage of their development, and that the growth of the integuments alone is emphasized (Fig. 1*c*). The stamens are perfectly regular and the anthers contain normal pollen grains.

(ii) The inflated carpels mentioned above open out into leaf-like structures and the traces of the ovules are lost (Fig. 1*d*). The anthers, however, appear to be normal externally, but contain a very large percentage of sterile pollen grains. The other floral parts are approximately at the same stages of transformation as in (i).

(iii) The carpels of the ovary are completely leaf-like and are borne on fairly long *petioles* springing from a common stalk, which corresponds to the fleshy gynophore of the previous stages (Fig. 1*e*). The anthers, while continuing to be normal externally, show a high degree of virescence with the development of the stomata and the chlorophyllous tissue. The anther locules are reduced to narrow spaces (Fig. 5*i*) and the pollen grains are all sterile. The petals are more leaf-like, both in contour as well as in venation, than in the preceding stages.

(iv) An extreme stage of the abnormality of the flower is that where the ovary is replaced by a foliar shoot (Fig. 1*f*). The anthers begin to show all external features of phyllody, the bases of the anther lobes spreading out as green expansions (Fig. 1*h*, *i*). The anthers are thus the last of the floral organs to become phyllodes.

Trichosanthes anguina (snake gourd). The male flowers, where the petals are completely transformed into leaves, show various degrees of phyllody of the anthers, and the most complete stage is that of an extensive foliar shoot springing from the centre of the flower (Fig. 2*e*). The sinuous anthers pass successively through a series of changes, the anther loops loosening gradually and the connectives becoming flattened more and more to form leaf-like structures (Fig. 2*c*, *d*). The anther locules are correspondingly reduced and produce abnormal pollen grains. Even in the early stages of the phyllody of the anthers, the stomium is absent and the endothecium loses its characteristic fibrillar nature. The chlorophyllous tissue which begins to develop at this stage with the formation of the stomata (Fig. 2*c*) becomes more conspicuous and extensive in succeeding stages of phyllody.

The male flower seems to be always in advance of the female flower in so far as the formation of an axial foliar shoot is concerned, because the ovary is already degenerate even in the normal male flowers. In the female flower, on the other hand, the initial stages of

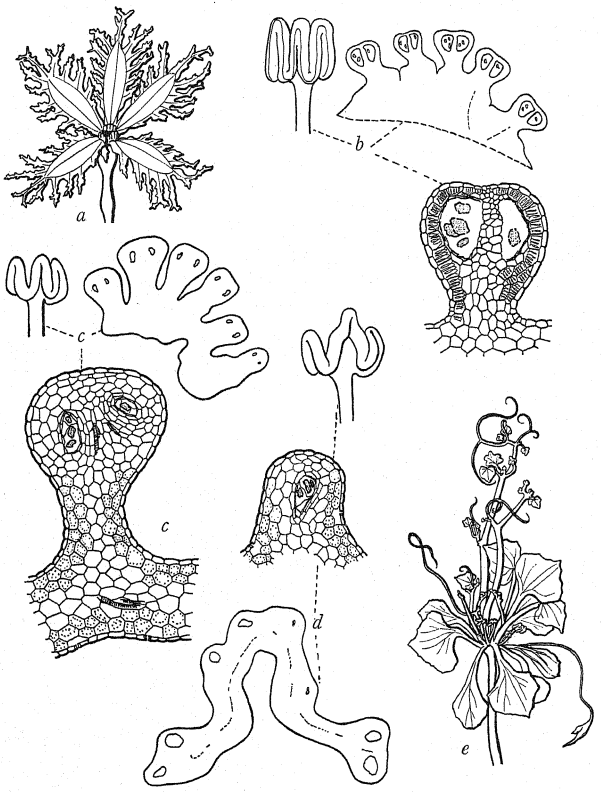

Fig. 2. *Trichosanthes anguina*. *a*, early stage in the abnormality of a male flower, petals leaf-like but the anthers normal; *b*, another of same with section and part enlarged; *c* and *d*, stages in the phyllody of the anther with sections enlarged; *e*, male flower with a well-developed axial foliar shoot.

the formation of a foliar shoot would be the disappearance of the ovules on the placental ridges, which then are thrown into a number of folds (as seen in a transverse section). The folds of adjacent ridges fuse together and form a central axis which proliferates into a shoot.

Allamanda grandiflora. With the transformation of the petals into phyllodes, the ovary is replaced by a fairly elongated axis, which bears terminally a pair of leaf-like structures, the carpels. The anthers, which are normal externally, become massive on account of an increase in the size of the connectives. The anther locules are reduced in size and contain a very few sterile pollen grains (Fig. 3*c*). The wall of the locules contains the chlorophyllous tissue with stomata in the epidermis. The fibrillar nature of the endothecium is lost, while the stomium may persist to some extent. In one or two abnormal flowers, axillary buds were found in the axils of the petals (Fig. 3*b*). The vascular strands for the buds are continuous with the main vascular strand that passes into the "gynophore" of the leafy carpels.

The most interesting abnormality was seen in the ovaries of only two flowers. In these, the axis of the abnormal ovary bears distally two carpels, which separate and tend to grow out into two leaf-like structures (Fig. 3*d*). Higher up, the two carpels again meet, bear a number of ovules and form a short style and a large stigma. The ovary in these abnormal flowers is, therefore, an open one, and the ovules are clearly visible from the outside on account of the separation of the carpels at the base of the ovary.

In one of the abnormal ovaries mentioned above there is a single terminal vegetative bud between the two carpels (Fig. 3*e*). The vascular supplies to this bud are in continuation with the vascular cylinder in the gynophore-like axis below the level of the separation of the carpels. The vascular strands of the carpels are also connected on to this main cylinder. In the other abnormal ovary, there are two vegetative buds within the carpels (Fig. 3*f*, *g*). These two buds are derived by a splitting of the apical portion of the gynophore-like axis. The ovules which are borne on the partially opened semi-foliar carpels of these ovaries seem to develop normally (an ovule at the megaspore mother cell stage is represented in Fig. 3*h*), but whether they continue to form fully developed embryo sacs must for the present remain an open question, as more material for study could not be procured.

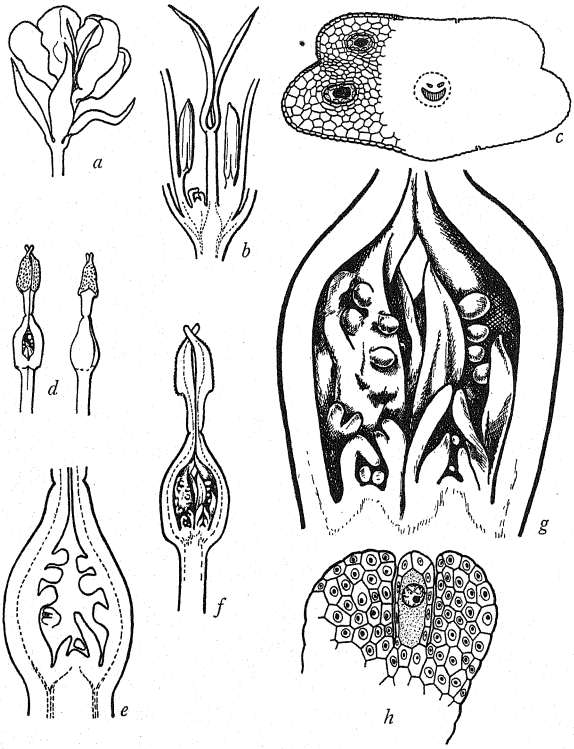

Fig. 3. *Allamanda grandiflora*. *a*, flower with a pair of "leaves" in the place of the ovary; *b*, longitudinal section of flower showing apparently normal anthers and an axillary vegetative bud; *c*, transverse section of anther in *b*; *d*, two views of an abnormal (open) ovary; *e*, longitudinal section of an abnormal ovary with one vegetative bud terminally; *f*, same as above with two buds; *g*, same enlarged to show ovules; *h*, an ovule from above at the megaspore mother cell stage.

PETALODY

The phenomenon of petalody is described here in only one case. In the abnormal flowers of the yellow variety of *Jasminum* (Fig. 4*a*), the sepals are fewer in number but larger in size than in the normal flowers. The ovary is perfectly normal and the only condition of abnormality is seen in the transformation of the anthers into petaloid structures (Fig. 4*b*). The two anthers of a flower may show identical or different degrees of petalody. In the earlier stages, while one of the anther lobes of a stamen may be completely formed into a petal, the other lobe may remain perfectly normal and produce regular pollen grains (Fig. 4*c*). In advanced stages of petalody, the connective is broadened out and the anther locules are greatly reduced (Fig. 4*d*) or completely absent.

In a solitary case of a flower showing petalody of the anthers, one of the petals contained a very small accessory flower in its axil. This accessory flower had only four petals, while the other floral organs were missing (Fig. 4*e*). The exact relationship of this flower to the parent flower could not be studied for want of more material, and a further search for similar instances was of no avail.

Utricularia coerulea. While the author was engaged in a study of the development of the gametophytes and embryo in *U. coerulea*, a solitary case of an abnormal ovary was accidentally discovered during an examination of the slides. The massive placenta shows here a splitting into two portions, one immediately becoming leaf-like (Fig. 4*f*), while the other, after a second splitting a little later, forms a reduced placenta and a second leaf-like structure (Fig. 4*g*). The placenta bears a few ovules, which contain regularly developed complete embryo sacs (Fig. 4*h*). The apex of the placenta, which in the normal ovary is only slightly elevated, is here prolonged extremely and passes in the stylar canal which is thus blocked. Therefore, the pollen tubes may perhaps be prevented from entering the ovary through their usual channel in the style and the ovary may fail to set seeds in the abnormal flowers. Since externally there is absolutely no indication of the abnormality, it is impossible to pick out the abnormal ovaries for study and hence a further detailed examination could not be undertaken.

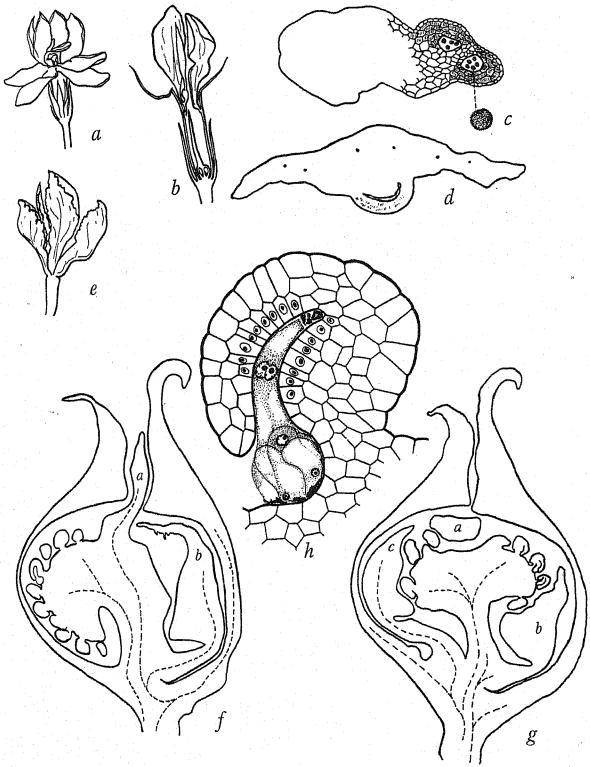

Fig. 4. *a–e*, *Jasminum*. *a*, flower with petalody of stamens; *b*, longitudinal section of same; *c* and *d*, sections of the two petaloid stamens at different stages; *e*, accessory flower. *f–h*, *Utricularia coerulea*. *f* and *g*, longitudinal section of abnormal ovary at different planes to show the leaf-like structures formed by the splitting of the placenta and a few ovules; *h*, one ovule of above enlarged to show the completely developed normal embryo sac.

VASCULAR ANATOMY OF THE ABNORMAL FLOWERS

During the course of this study, it was thought that an examination of the vascular anatomy of the abnormal flowers would perhaps throw some light on the nature of these abnormalities, and the following account is therefore included. In all cases the vascular strands that enter the different floral parts are similar to those in normal flowers. In *Tropaeolum majus*, after the formation of the vascular supplies to the sepals and the stamens, three large strands pass into the base of the ovary, where each splits into an inner and an outer portion (Fig. 5). The inner portion runs in the axis of the ovary and is exhausted below the style. The outer portion splits into a number of small strands which run in the ovary wall. The separation of these smaller strands is similar to the formation of a number of strands in the ordinary leaves. In the anthers also, with progressive phyllody, the number of strands increases from one in the very early stages, to several later on (Fig. 5*i*).

In the female flowers of *Trichosanthes anguina* showing phyllody, the ovary has three larger outer strands and a group of smaller inner ones arranged triradiately (Fig. 5). Later on, these smaller strands split further and are gradually lost, while the three outer larger ones increase in size (Fig. 5*l*, *m*), and when the foliar axis is formed by the fusion of the placental ridges become more or less continuous to form a ring, which closely resembles the vascular structure of the ordinary vegetative axis.

In the peculiar abnormal ovary of *Allamanda grandiflora*, the vascular strands are numerous and form a ring in the gynophore-like stalk of the ovary (Fig. 5*q*). When the carpels separate the entire ring divides into two halves, which pass into the two carpels (Fig. 5*r*). Higher up the number of strands in each carpel is reduced to three. one median dorsal and two lateral ventrals. The median dorsal strand disappears in the style and the lateral ventrals fuse and give rise to a stylar strand (Fig. 5*u*). This strand is greatly expanded in the stigma. The separation of the carpellary strands is similar to the separation of a leaf strand in the vegetative portions of the plant. The only difference perhaps is, that while in the case of a leaf a single large trace is separated from a well-developed continuous vascular ring of the vegetative axis, in the case of the ovary a number of smaller strands are separated to supply the carpels.

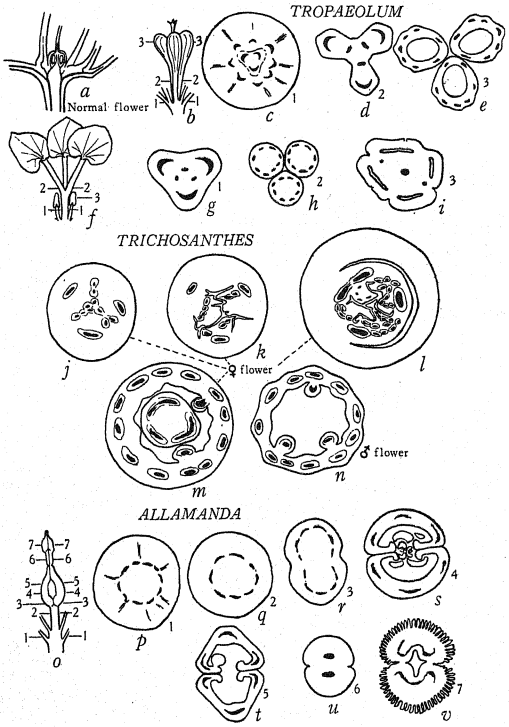

Fig. 5. The vascular structures in the various abnormal flowers. At *a* is shown the vascular strands in a longitudinal section of a normal flower of *Tropaeolum majus* for comparison. The numbers in each series indicate transverse sections at successively higher levels.

DISCUSSION

The abnormal features of flowers described here are regarded as reversions in which there is a reappearance of ancestral characters. The phenomenon of petalody may suggest that the members of the adjacent floral whorls show sometimes a certain amount of indefiniteness, namely, whether they should form one or the other floral part in the course of the evolution of the flower. The formation of an axial foliar shoot from the flower is an extreme case of the transformation of a flower, the initial stages in this being virescence and phyllody. But by reversions to an ancestral condition it is not even remotely suggested that the floral parts are in any sense metamorphosed foliar appendages as envisaged by Goethe so long ago.

The different floral organs do not show the same degree of mutability, and from the examples cited in this paper it is evident to some extent that the outer two whorls, those of the calyx and the corolla, which are already more leaf-like (more particularly the whorl of the calyx) than the other two inner whorls even in the normal flowers, show the stages of transformation less strikingly than the androecium or the gynoecium. The least susceptible to phyllody is the androecium, for it is only after all the other floral parts are affected that it shows signs of transformation. In the earlier stages it is externally perfectly normal for all purposes, though in its contents, the pollen grains, a certain degree of sterility may set in. It becomes foliaceous only after the innermost whorl, that of the carpels, has proliferated into a foliar shoot. The floral organ that is affected earliest is the gynoecium, because the floral parts constituting it, the carpels, are the nearest placed to the growing point of the floral shoot and the latest formed lateral organs on the floral axis.

The presence of a gynophore in the abnormal flowers of *Argemone mexicana* is stated by Joshi (1933) to be of phylogenetic importance, and he states that the development of this structure emphasizes the relation of the Papaveraceae to the Capparideae. On the other hand, a gynophore-like stalk is present in all instances of abnormality of the ovary mentioned in this paper, and this structure may be held to be of general occurrence in an ancestral condition of the ovary.

Accessory flowers have been described in *Nasturtium officinale* by Halket (1932) and Arber (1931). The latter states that it is difficult to interpret the relation of the accessory flowers to the parent flower, but suggests that they cannot be described as axillary to the petals

and that the petal plays the part of an axis rather than a leaf. The accessory flower in *Jasminum* may be regarded as axillary to the petal, which is considered to be of the nature of a leaf. Similarly the presence of a vegetative bud between the floral axis and the petal in the abnormal flowers of *Allamanda* may indicate an identical morphological nature for the petals.

The point of view from which the author regards the abnormalities here described is conveyed by the following statement of Worsdell (1915): "In very many cases the so-called 'freaks' and 'monstrosities' represent reversions or harkings-back, in one form or another, to an ancestral condition, but this will always take place in a way which is modified by the structure and idiosyncrasies of the organ which is undergoing change. For example, a vegetatively proliferated rose, whose floral organs change into green leaves and become vertically displaced owing to the elongation of the floral axis, tells us better than the facts of its ontogeny would, better than those of its anatomical structure, better even than any comparison of the flower in its normal state with any other type of flower, that the flower has been derived in the past by congestion and abbreviation of an axis, and by the extreme reduction and modification of leafy sporophylls. But it would be absurd to suppose that the leafy shoots from which our flowers originally sprang in any sense resembled, save in the matter of possessing an elongated axis and leafy sporophylls, those into which our modern flowers so frequently proliferate. Under special conditions of nutriment and moisture, the older tendency to break the bonds which an adaptive evolution has placed upon the flower becomes manifested."

Acknowledgements

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GRAPHIC KEYS FOR THE IDENTIFICATION OF SPHAGNA

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(With 5 figures in the text)

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Introduction

THERE are probably a large number of ecologists and field botanists who find the need for some acquaintance with the *Sphagnum* genus from time to time. The literature on the subject is rather unwieldly, much of it is written in foreign languages and it is often difficult to obtain and inconvenient to handle. In general, too, only after considerable experience with the whole genus can species be recognized rapidly in the field or laboratory. The following graphic keys are based on a generalized system of *Sphagnum* classification, and they purpose no criticism of individual systems. They are intended, not as an infallible guide to the diagnosis of "difficult" species (for which classical monographs must always be consulted), but for placing the main body of a field collection in a systematic scheme.

The experienced taxonomist rarely runs through a verbal key before determining a familiar species; he sums up his optical impressions at once, and compares them mentally with the appearance of some well-known "type". The field ecologist, on the other hand,

has little time or opportunity for assembling a full type collection. Since he works probably with only a limited number of members of the genus, the meaning of comparative terms—even in English descriptive texts—remains largely a matter of guesswork. Ambiguities and difficulties in translation lead constantly to a consultation of the illustrations, and these are often of a very diagrammatic nature.

By introducing a compact arrangement of realistic drawings, the author hopes to avoid some of these disadvantages, and to give at a glance the general characters for which the ecologist has, formerly, had to rely on experience alone. Once the conventions used in the graphic keys have become familiar, the drawings should call up the sort of picture that is seen with the microscope during any examination. They will then perhaps serve as a rough and ready pocket type collection, and save time in the naming of typical samples.

The material from which the plates were made consisted of some 450 preparations, chiefly from herbarium material belonging to the Växtbiologiska Institution of Uppsala, Sweden. The specimens were selected for their authoritative naming and reliable habitat records (much of the material having been obtained by exchange from the collections of Jensen, Lindberg, Warnstorf, etc.). The herbarium samples have since been generously supplemented by specimens from the personal collections of Prof. Du Rietz and Prof. Osvald, and come from Scandinavia, the British Isles and New Zealand. Other specimens were collected in North-west Europe by the author.

The nomenclature and specific status given to the Sphagna illustrated here, represented the unpublished views of Prof. Du Rietz in 1936–7. Apart from the group status of *S. subsecundum*, *S. pylaiai* and *S. ångströmii*, the present arrangement corresponds fairly well to Le Roy Andrews's recently revised system.[1] Some nine "species" not recognized in Le Roy Andrews's paper are depicted for convenience of reference to earlier literature, while a further three still maintained in the British Census Catalogue are omitted as of little significance to the ecologist.

The general separation of the genus into groups has not been treated graphically because of the difficulty of illustrating non-specific characters. For convenience of reference, and as an aid in field work, diagnostic group characters recognizable with a hand-lens are arranged in a text key immediately preceding the plates. It

[1] Differences are noted in the list of synonyms on p. 11.

has seemed to the author unsatisfactory to attempt to exclude microscopic characters from the key entirely, and it may be necessary in difficult cases to rely on such characters altogether.

Before closing this introductory section the author would like to express her sincere gratitude to Prof. Du Rietz of Uppsala University in whose laboratory she was privileged to work for several months. It was at his suggestion and with his participation that the technique was elaborated and the groups revised. It was only through his wide taxonomic experience and linguistic capacity that the scope of the present contribution could be made representative of general European *Sphagnum* systematics.

Note on the main subdivisions of the genus

In most instances separation of the Sphagna into the subgenera *Inophloea* and *Lithophloea* is quickly accomplished in the field. Similarly the divisions of the *Lithophloea* (the Warnstorf "groups" are generally recognized after a little practice without microscopic examination. For the general ecologist, a key is appended which summarizes a number of useful field characters in a compact form.

Unfortunately, the macromorphology of Sphagna can very rarely be regarded as of accurate diagnostic value, even though this is responsible for the characteristic "look" of species in their natural habitat. The general appearance of the living *Sphagnum*, indeed, not only attracts the eye of the collector but is the very basis upon which the ecologist builds up an impression of distribution, indicator significance, etc., in the field. An attempt is therefore being made to summarize certain aspects of the general macromorphology of the group which does not lend itself to the closer definition required by a key.

Very broadly, the *Palustria* (*Cymbifolia*) group may be described as coarse and vigorous, with a characteristic "hooded" leaf; the *Acutifolia* are delicate, pinkish, regular, cushion-forming; the *Cuspidata* with tufted heads, sharply rolled and somewhat drepanoclade leaf, often aquatic or damp-loving; the *Squarrosa* with pointed branchlets and often recurved leaf, growing only in eutrophic situations; *Rigida* with soft, untidy habit, in loose cushions; *Subsecunda* most variable of all, with loose, often coarse growth, sometimes lax, gelatinous, and aquatic, sometimes irregularly compact, with branchlets crowded, stem often clothed with large leaves.

In colour, the genus in the living state displays every shade from purple-pink, crimson and russet, through yellow, yellow-green and olive-green, to a greenish or purplish brown, or even black. The first three tints appear only in the *Acutifolia*, and in *Sphagnum magellanicum* of the *Palustria*; the last only in submerged *Subsecunda*. An orange-yellow coloration is typical of certain *Subsecunda*, but appears also in the *Squarrosa* (*Sphagnum teres*) and in *Rigida* (*Sphagnum compactum*). The remaining Sphagna tend to be green or yellow-green, although they may assume a delicate straw colour or deep olive-green in certain conditions. Green specimens of almost every typically coloured *Sphagnum* are known.

The arrangement of the groups in the plates is determined rather by space than considerations of natural affinity, since the taxonomic problem of group status lies outside the scope of this paper. Reference should be made to recent publications by Le Roy Andrews (1936) and Åberg (1937) for details of taxonomy.

For example, the Warnstorf group *Subsecunda* is reduced by both these authors to specific rank. For correlation with earlier nomenclature, those Warnstorf *Subsecunda* species are illustrated as varieties of *Sphagnum subsecundum* if recognized by Åberg (1937). The *Sphagna crassicladum* and *camusii* and *Sphagnum turgidulum* of the British Census Catalogue are omitted as extreme modifications of *Sphagnum subsecundum* var. *bavaricum* and var. *rufescens* respectively. [*Sphagnum subsecundum* var. *rufescens* (*sensu* Warnstorf) which has recently (Sherrin, 1937) been struck off the British Census Catalogue is still recognized by Åberg as a south-east Swedish species. The author has not examined any satisfactory material of this *Sphagnum*, so that the illustration has been based on the identity of Åberg's description of the branch-leaf pore structure with that for *Sphagnum subsecundum* var. *bavaricum* which was drawn from preparations.] *S. holtii* and *S. obesum* of the British Census Catalogue are regarded by the author as coming very close to *S. subsecundum* var. *inundatum*. *S. pylaeai* is not regarded by Le Roy Andrews as a variety of the old *Subsecunda*, but as an independent species within the *Cuspidata* "series".

As to the *Cuspidata* group of the British Census Catalogue the present keys make two omissions: *Sphagnum fallax*, which seems to differ little in both taxonomic and ecological range from *S. recurvum* = *S. apiculatum* of the key; *S. torreyanum*, a species evidently nearly related to *S. cuspidatum*.

In the *Acutifolia*, *Sphagnum subtile* and *S. tenerum* of the British Census Catalogue, are not illustrated, as differing little from *S. acutifolium*. On the other hand a *S. angermannicum* is depicted which is probably identical with *S. molle* var. *limbatum* of the British Census Catalogue. From very limited material this *Sphagnum* has seemed to the author to merit recognition as a separate species.

The monotypic group *Truncata* is now fused by Le Roy Andrews with the *Squarrosa*, which is illustrated here on another plate.

The revised *Lithophloea* series requires closer inspection than the Warnstorf group, with very little corresponding improvement in the ease of recognition.

A GENERAL KEY

Based on branch leaf characters visible with the hand-lens

A. Branch leaves (B.L.) *cucullate* (hooded) cortical cells with spiral fibrils
Subgenus *Inophloea* = *Palustria* (*Cymbifolia*)

B. B.L. *non-cucullate*: no spiral fibrils—toothed or truncate at apex
Subgenus *Lithophloea*

I. B.L. *broadly truncate*, large, very revolute margins

(*a*) B.L. with resorption-furrow ... ... *Rigida*

(*b*) B.L. without resorption furrow
(i) Stem leaves smaller than B.L. *Truncata* (*S. ängströmii*)
(ii) Stem leaves larger than B.L. *Acutifolia* (*S. angermannicum*)

II. B.L. *not broadly truncate*, acute or toothed at apex

(*a*) B.L. *broadly ovate*. Chlorophyllose cells not triangular. (Colour never red)

(i) B.L. obtuse, very revolute margin ... ...*Subsecunda*

(ii) B.L. truncated cuspidate less revolute ... ... *Squarrosa*

(*b*) B.L. *narrowly ovate*. Chlorophyllose cells triangular with convex (ventral) exposure. (Colour never red)
Cuspidata, series *Ovalia* (*S. tenellum*)

(*c*) B.L. *ovato lanceolate-lanceolate*, acute. Chlorophyllose cells triangular-trapezoid

(i) B.L. *somewhat undulate or crispate*; with greater convex (dorsal) exposure. Stem leaves very small
Cuspidata, series *lanceolata*

(ii) B.L. *neither undulate nor crispate*; chlorophyllose cells with greater concave (ventral) exposure. Stem leaves relatively larger than in (*c*) (i)

(α) Very numerous branchlets in each fascicle
Polyclada (*S. wulfianum*)

(β) Generally fewer than 7 branchlets per fascicle *Acutifolia*

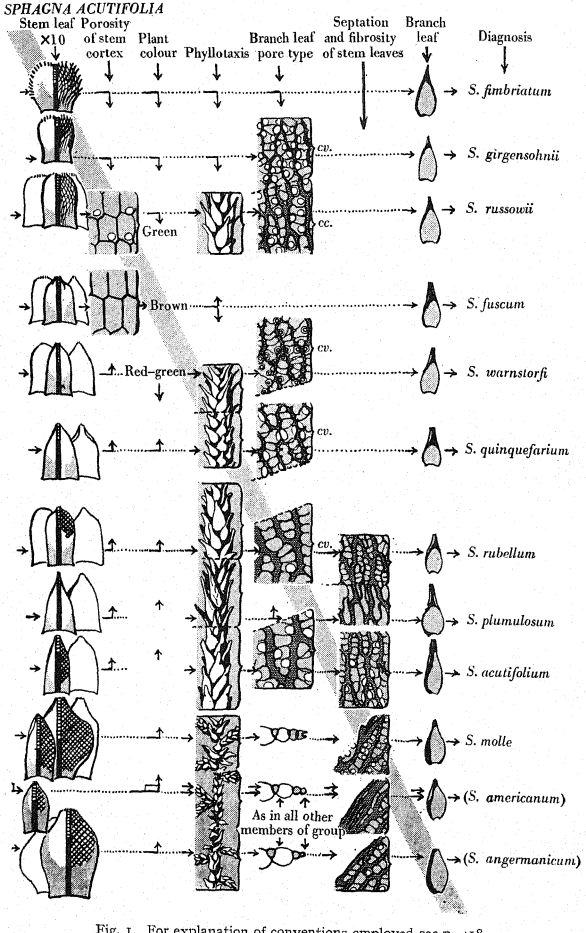


Fig. 1. For explanation of conventions employed see p. 418.

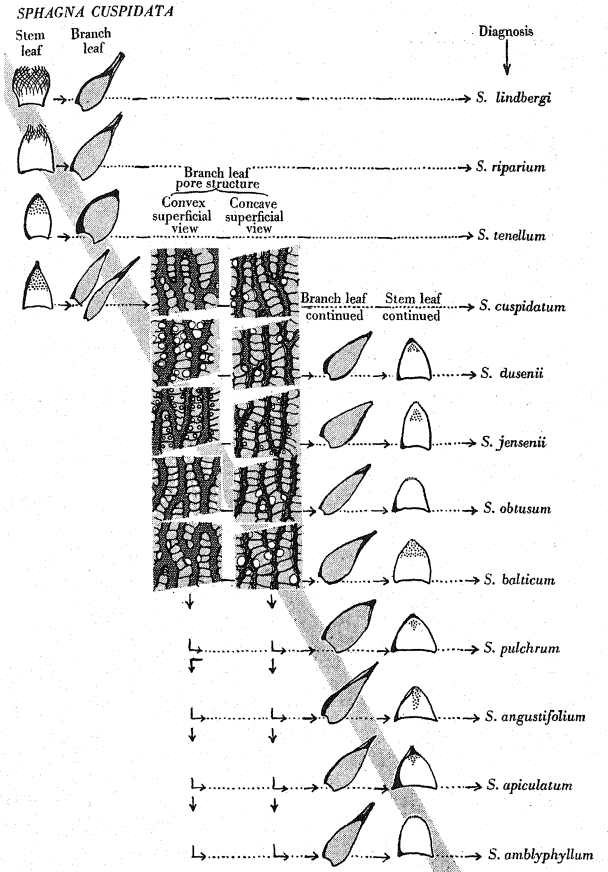


Fig. 2. For explanation of conventions employed see p. 418.

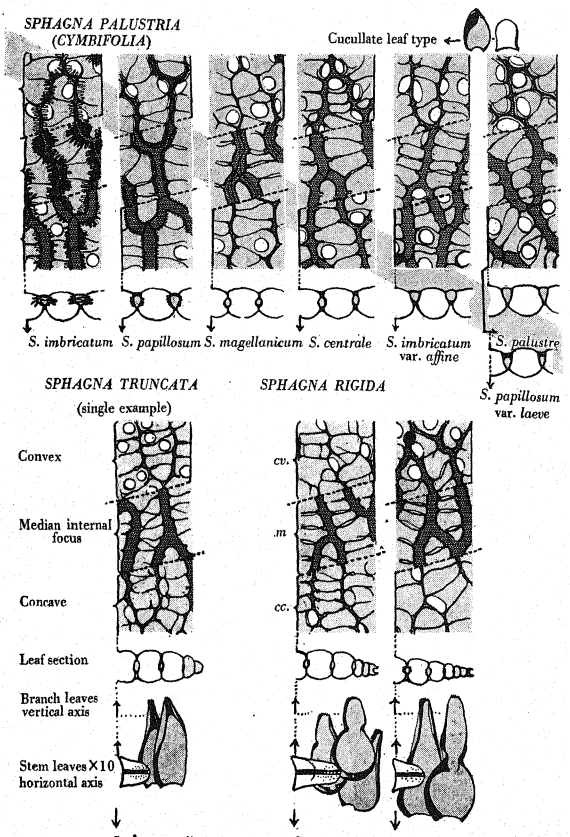
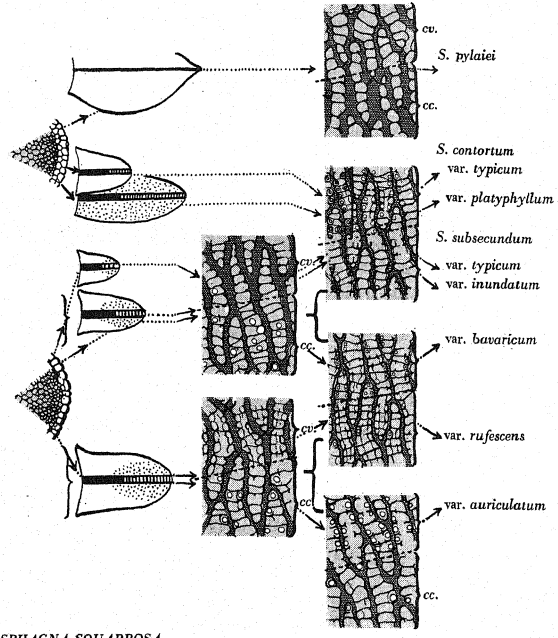


Fig. 3. For explanation of conventions employed see p. 418.

SPHAGNA SUBSECUNDA

Stem section. Stem leaf×10. Stem leaf×200. Branch leaf×200.



SPHAGNA SQUARROSA

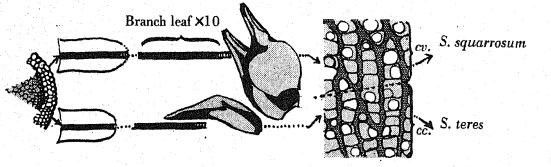


Fig. 4. For explanation of conventions employed see p. 418.

Explanation of conventions used in graphic keys

Stem leaves are in black and white outline.

Fimbriation is depicted realistically.

Fibrillation is indicated by hand stippling.

The extent of purely chlorophyllose tissue (without hyaline interruptions) is suggested by half-tone shading.

The schematic "mid-rib" indicates the usual amplitude in size for well grown-leaves, and follows Warnstorf's figures at a scale of × 10.

Branch leaves are in half-tone shading.

They represent the form most usually found proximally on up-turned branchlets (see preparation technique). Scale is not given, since size is rarely diagnostic.

Phyllotaxis is shown by the view through a low power binocular microscope with exaggerated illumination and relief.

Details of pore arrangement and structure are drawn at various scales to suit the minuteness of detail required. When the leaf is observed by superficial focus from the *convex* side, the sketch is always truncate obliquely *below* the rectangle—if from *concave* side, the sketch is obliquely truncated above (the two views fitting together, when both are shown, along a dotted line to indicate deepening of focus on the same material). When a median internal focus is required this is depicted always as a quadrilateral between the upper and the lower focus views (*S. palustria*).

Leaf sections are depicted realistically from typical material.

Deep shading is used only for deep-staining chlorophyllose cell structure.

Half-tone is used as indicated above, and for all parts of the microscopic structure which retains a light stain.

Pores are indicated as white openings in the half-tone ground; *outlined* if they are distinct, *ringed* if they are bordered, or *left as perforations* if faint (as in *S. obtusum*).

Pseudopores (of equal diagnostic value in certain groups) are indicated as imperforate outlines on the half-tone ground.

List of authorities and synonyms

SPHAGNUM (Dill.) Ehrh.

**acutifolium* Ehrh. = †*capillaceum* (Weiss) Schrank
var. *subtile* (Russ.) Warnst.
var. *tenerum* (Aust.) Warnst.
**americanum* Warnst.
**angermannicum* Melin
**†angströmii* C. Hn.
**apiculatum* (H. Lindb.), see *recurvum*
**†balticum* Russ.
**centrale* C. Jens.
**†compactum* DC.
**contortum* Schultz
**var. *platyphyllum* (Sull.) Åberg
**†cuspidatum* Ehrh.
var. *torreyanum* (Sull.)
**†dusenii* C. Jens.
**†fimbriatum* Wils.
**†fuscum* (Schimp.) Klinggr.
**†girgensohnii* Russ.
**†imbricatum* (Hornsch.) Russ.
**var. *affine* (Ren. et Card.) Warnst.
†**jensenii* H. Lindb.
**†lindbergii* Schimp.
**†magellanicum* Brid.
**†molle* Sull. = *tabulare* Sull.
**†obtusum* Warnst.
**palustre* L. = †*cymbifolium* Warnst.
**†papillosum* Lindb.
**var. *laeve* Warnst.
**†plumulosum* Röll.
**†pulchrum* (Lindb.) Warnst.
**†pylaiei* Brid.
**†quinquefarium* (Lindb.) Warnst.
†*recurvum* Palis. = *apiculatum* (H. Lindb.)
**var. *angustifolium* C. Jens.
**var. *amblyphyllum* Russ.
var. *fallax* (Klinggr.)
**†riparium* Ångstr.
**rubellum* Wils.
**russowii* Warnst. = †*robustum* (Russ.) Röll.
**†squarrosum* Pers.
**†strictum* Sull.
**†subsecundum* Nees
**var. *auriculatum* (Schimp.) Lindb. = var. *obesum* (Wils.) Warnst. *ex parte*
**var. *bavaricum* (Warnst.) Åberg = var. *crassicladum* Warnst. *ex parte* = var. *camusii* (Card.) Warnst.
**var. *inundatum* (Russ.) Åberg = var. *obesum* (Wils.) Warnst. *ex parte* = var. *holtii* Warnst.
**var. *rufescens* (Br. germ.) Åberg = var. *turgidulum* (Warnst.)
**†tenellum* Pers. = *molluscum* Warnst.
**†teres* (Schimp.) Ångstr.
**†warnstorfii* Russ.
†*wulfianum* Girg.

* Figured in the graphic keys.
† Recognized in Le Roy Andrews's revised system (1936).

THE USE OF THE GRAPHIC KEYS

Apart from that for the *Sphagna subsecunda*, the keys are all constructed on the same plan, and are intended to give at a glance all those characters (other than growth form) which are relevant for the quick determination of any *Sphagnum* species. The keys are arranged so that each feature illustrated is part of a series leading finally to a specific name. These series are arranged to be followed from left to right in all groups, except *Palustria* (*Cymbifolia*), *Truncata*, and *Rigida* (Fig. 1) where they are placed *down* the page on account of space. The following explanation applies particularly to Figs. 1 and 2; an additional note on Fig. 4 appears later.

In different groups, of course, different characters are of diagnostic importance, so that the same features do not necessarily appear on every plate. Within each group, however, all characters of diagnostic value for recognizing any specimen are illustrated in the vertical columns in their full range. A survey of these columns provides the variation amplitude of a number of diagnostically useful features within each group. The horizontal lines connect the specific manifestations of each of these characters in turn.

The vertical order of the species list is decided as far as possible by the ease of diagnosis, so that the investigator may dispense with readily determined species early in the key. If a specimen can be recognized at low magnification on a single character, it will generally appear towards the top of the list, and the character will be illustrated towards the left-hand side of the page. If the species is only identifiable after the confirmation of a number of characters, it will be found towards the bottom of the page. The final separation of this species from adjacent members of the list will then depend on a character illustrated towards the right-hand side of the page.

A diagonally sloping half-tone band is drawn to cross the successive columns just where each character becomes of specific diagnostic importance; so that, except for confirmatory information it is seldom necessary to consult characters to the right of this band. If, moreover, a specimen suspected of belonging to a familiar species fails to exhibit the character emphasized by the half-tone band, it is advisable to start following the key at once (from the top left-hand corner, and working across the lower triangle from left to right).

The vertical system of arrows is used to avoid a repetition of drawings. They act as "ditto" marks below the lower drawing in each column and above the uppermost. The horizontal arrows merely

direct the eye across the columns to the appropriate specific name. Where more than one horizontal row of arrows passes across a single drawing (see Fig. 4), that drawing is applicable to both or all the species indicated; where the row crosses a perpendicular arrow (see Fig. 1), the appropriate illustration will be found in the direction of that arrow.

By following these systems of arrows one may build up a more complete picture of the species than is necessarily required for its identification. The vertical columns also serve, by presenting the range of variation within the group, to explain the meaning of such relative terms as "pores numerous", "pores large", "pores bordered", etc., which offer so much difficulty to the investigator possessing only a limited acquaintance with the group. On the other hand, it must be emphasized that the drawings can only depict what have seemed to the author to be fairly representative types. They are not intended to exclude minor divergences, but rather to indicate graphically the nature of the differences upon which the keys have been based.

Suppose, for example, a member of the *Sphagna acutifolia* awaits identification. If its stem leaves are of the *fimbriatum* or *girgensohni* type, the species, as the half-tone band indicates, requires no further confirmation of its identity; but suppose it to possess a spatulate stem leaf, and the possibility of its being a variant of several species arises: the porosity of the stem cortex will then decide whether or not it is *S. russowi*. A marked chestnut-brown coloration is completely diagnostic for *S. fuscum*; five-rowed phyllotaxis is generally a good character for recognizing *S. warnstorfi* and *quinquefarium*—and these are then separated immediately on stem or branch leaf form, with the additional check of leaf-pore structure in more doubtful cases. As one works through the key, the species characters become less easy to define and more data are required for making a determination. However, if the individual characters be noted in the order suggested by the headings at the top of the figures, one may be confident that no character is being examined unnecessarily even as regards the rapid determination of the species.

For speed and convenience in making identifications, macro-characters have, wherever possible, been dealt with first; so that a number of possibilities may be adopted or discarded after hand-lens examination, as in the early parts of the *Acutifolia* and *Cuspidata* keys. If lens diagnosis fails, the keys next take one on to micro-scopic characters of increasing subtlety (although macro-characters

may again be called upon to supply confirmatory evidence). Unfortunately, in the *Sphagna palustria*, there are no useful lens characters of diagnostic importance. *S. imbricatum*, however, may be detected with the lowest powers of the microscope; *S. papillosum* requires rather more minute determination; *S. magellanicum*, if not of the typical reddish colour, and *S. centrale* must be examined by the "differential focus" technique. (The author regards section-cutting as unnecessary for the first four species of this group, and only useful for extra confirmation in *S. imbricatum* var. *affine*.) The difference between *S. palustre* and the variety *laeve* of *S. papillosum* can of course only be detected in the plane at right angles to the leaf surface (sections therefore are necessary only in separating the last two species).

With regard to the *S. subsecunda*, Fig. 4, the method of presentation is necessarily rather different on account of the complex character of the key. Since the group has been revised by Åberg as recently as March 1937, it has seemed appropriate to follow his classification scheme. The sketches, though made independently, and from different material, have therefore been arranged as illustrations to his key. No single system of characters can be recommended for diagnosis within this group; the species differences are generally based on character aggregates or are of a statistical nature. The half-tone band convention is therefore not employed here. This key is read from left to right, beginning with the stratification of the stem cortex, and differentiating next on stem leaf size, and later on relative pore frequency of the leaf faces.

The *S. squarrosa* are arranged in the same columns merely to maintain uniformity on the figure; if space had permitted, they would more fittingly have appeared beside the *Truncata* and *Rigida* groups.

A TECHNIQUE FOR THE PREPARATION OF REFERENCE MATERIAL

Write duplicate labels for slide and test-tube. Heat water-bath.
Select a branched specimen of *Sphagnum* sample.
Remove one branch, and mark the remaining as "type herbarium material".
Place in warm KOH solution (all unused test-tubes and water and KOH bottles stand in a water-bath).
Bring to boil and boil for a few seconds.
Transfer liquid to another vessel.
Add hot water and boil again.
Rinse and boil again.
Rinse in cold water.
Rinse in alcohol.
Transfer branch to large glass dish of dilute alcohol.
Strip off branchlets from one (vertical) half of the stem if they grow densely.

Select six vigorous upturned branchlets and, if necessary, three pendent branchlets.

Strip off and discard remaining fascicles.

Transfer stripped stem and the selected branchlets to staining medium—gentian violet in alcohol.

Leave in stain 3–6 min.

Remove the seven (or ten) objects from the stain.

Wash in dilute alcohol.

Wash in large vessel of distilled water.

Wash in large vessel of dilute glycerine.

Leave to stand in watch-glass of pure glycerine.

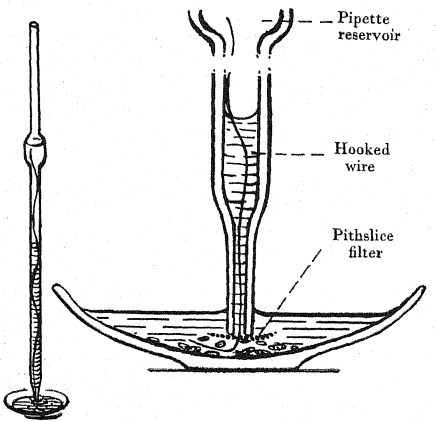


Fig. 5

Select a portion of stem and dissect off six stem leaves, together with a small piece of stem cortex.

Arrange objects centrally in a drop of pure glycerine* on slide (with the help of a binocular) so that the three leaves on the left-hand side lie concave side uppermost and vice versa.

Lower cover slip immediately.

Select an originally upturned branchlet and dissect off six leaves. (A number of leaves are stripped off with a fine scalpel and the larger, i.e. the proximal ones, selected.)

Arrange in a second drop of glycerine immediately below the label on the slide so that the three on the left-hand side lie concave side uppermost, and

* For semipermanent preparations the glycerine may be replaced by a hardening medium such as gum chloral, which makes the slides more convenient to handle although the stain is more apt to diffuse out.

vice versa. (Arrange a further six leaves from pendent branchlets below the former if required.)

Lower cover-slip immediately.

Select two or three uninjured, originally upturned, branchlets and the uninjured portion of stem; cut the stem into two parts.

Lay the objects in needle grooves in a pith cylinder—draw out the branchlets slightly so that the leaves come to lie flat against the stem.

Cut sections.

Place sections in water and remove the floating pith (the sections sink to the bottom).

Pierce a small pithslice with a hooked wire and push the wire up through the nozzle of a 5 c.c. pipette (see Fig. 5).

Draw up water gently until the pithslice filter lies against the mouth of the pipette.

Then filter the sections from water in the watch-glass by suction.

The lightest, therefore thinnest, sections adhere to the pithslice.

Pour off the excess water from the broader, open end of the pipette.

Suck the pithslice as free as possible from capillary water.

Float off sections into a drop of glycerine on the slide.

Remove larger pith fragments or thick sections (with the help of a binocular).

Lower cover-glass.

Sealing:

Heat a mounted needle until almost red-hot.

Dip into mounting wax and transfer the fluid wax to the slide.

The fluid wax should not at first come into contact with the cover slips, but form a raised border of even thickness about 1 mm. from them.

Next, take the hot needle and press the remelted wax towards the margin of the cover slip, so that it makes contact along the whole border.

Now follow the margin of the cover-glass with a very hot needle so that the wax runs some 0·5 mm. over the border and sealing is complete.

The outer border of the wax frame may be straightened by running the needle round the outer edge. Finally, the surface of the wax is smoothed (to prevent damage to or by microscope objectives during investigation) by holding the slide in a spirit flame for a moment. (Great care must be taken not to boil the glycerine or allow the cover glasses to slip out of place.)

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STRATIGRAPHY AND DEVELOPMENT OF TWO RAISED BOGS NEAR TREGARON, CARDIGANSHIRE

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(With Plate VI and 9 figures in the text)

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I. Introduction

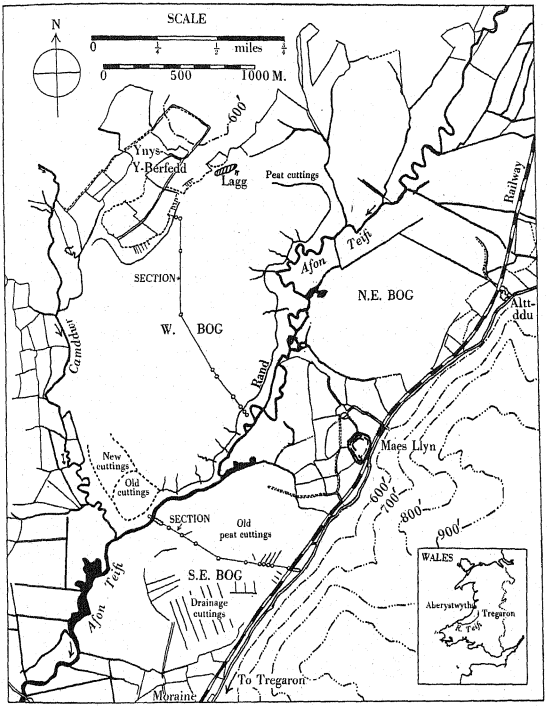
[Raised bogs are cushion-shaped accumulations of peat, formed mainly by the growth of *Sphagnum* moss. They are limited to regions with specific rainfall and evaporation conditions, and the bulk of the bog obtains no water other than that reaching it directly from the air. They are often found to have developed from a lake centre, which has become overgrown with fen peat, and then the acid *Sphagnum* peat has formed. Since the primary lakes are frequently of glacial origin, raised bogs have generally proved to be extremely satisfactory sources of long continuous profiles illustrative of post-glacial forest and climatic history. They have been freely exploited by Scandinavian pollen analysts, and it was in search of similarly satisfactory bogs that Tregaron was visited in 1936. Raised bogs are very rare in southern England and the Midlands, although they begin to be comparatively abundant on the Welsh border, in Shrop-

shire, and in Cheshire. In Wales, though information is sparse, they seem to be infrequent. However, the three bogs which lie in the Teifi valley at Tregaron are exceptionally good examples, and they have the great advantage to the pollen analyst of lying between the English and the Irish bogs, and promising therefore to be a link between the two countries. A cursory visit had already been made to these bogs by Erdtman (1928), who published a single profile from one of the two eastern bogs, and also took pollen samples, though the full diagram was not published.

In the field work, which took place during the first fortnight of July 1936, Dr K. B. Blackburn, Dr H. A. Hyde and A. G. North gave valuable assistance, and Dr Hyde has also contributed one of the pollen diagrams (diagram S.E. 10). During the stay at Aberystwyth Prof. Newton kindly placed the Botanical Laboratory in the University College at the disposal of the party, and for this and many other kindnesses thanks are due to Prof. Newton. The authors also wish to thank the other members of the field party for their help, without which much less would have been possible, and to express their gratitude to Lord Lisburne, who kindly gave access to his property].

A broad ridge of moraine runs across the valley of the river Teifi in the immediate vicinity of the village of Tregaron, which is placed on the margin of the moraine as it abuts against the steep side of the valley. Below this moraine, outwash gravels extend down the valley for a considerable distance. According to Charlesworth (1929) the moraine was deposited by a local glacier which had its origin in snow fields on the higher ground to the north. Its deposition he regards as contemporaneous with that of the "Newer Drift" of Great Britain and Ireland, but this correlation is open to some doubt.

The Teifi flows across this ridge, but above it the course is slow and meandering, and on either side of the river large raised bogs, known locally as Cors Goch Glan Teifi, have developed (see Text-fig. 1). The largest of the bogs lies on the west side of the river, here flowing approximately from north-north-east to south-south-west, and is roughly oval in shape. Its length is about 2400 m. (1½ miles) and its maximum breadth about 1200 m. (¾ mile). Two smaller bogs are on the eastern side of the Teifi, and are separated by a ridge of high ground which runs out toward the Teifi at Maes Llyn. The more northern of these two bogs has been extensively altered by drainage, but the south-eastern bog, which is about 1200 m. (¾ mile) long and 800 m. (½ mile) across, and the large bog across the river



Text-fig. 1. Sketch-map of the Teifi valley showing the meandering river and the three large raised bogs. Across the S.E. bog and the W. bog are shown the lines of section made by borings and profiles.

are relatively intact, though in places considerable cutting has taken place at the margins. A section was made across both these bogs, mainly by boring with a Swedish Djos drill[1] with chamber 50 cm. long and 3 cm. diameter, but where profiles were available in the cuttings these were also utilized. At certain borings samples were taken for pollen analysis.

The surface vegetation of the western bog was studied at the same time by another party, and will be described elsewhere.

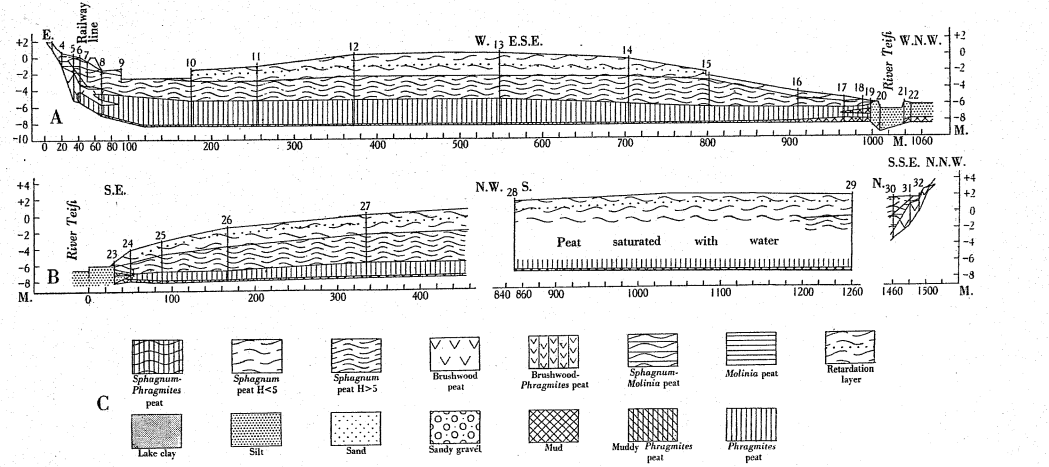
II. STRATIGRAPHY OF THE SOUTH-EAST BOG

The line of section in the south-eastern bog ran from its margin near Coed Treflyn (1⅛ miles north of Tregaron village), almost due west to the centre of the bog. Most of the margin of the bog had been extensively disturbed by the embankment along which the railway runs between bog and hillside, but at Coed Treflyn the disturbance was least, and here it was possible to include several peat cuttings in the section. From the centre of the bog the line turned west-north-west to approach the Teifi at right-angles. Twenty-two profiles or borings were made along the line of section, though not all of these reached the bottom of the bog, and at three points samples were taken for pollen analysis. The borings were serially numbered from east to west, and to each number the letters S.E. were prefixed. From the data thus obtained, the sections on Text-figs. 2 A and 3 have been drawn up. A datum for levelling purposes was established on the railway embankment at 538 ft. (160 m.) O.D.

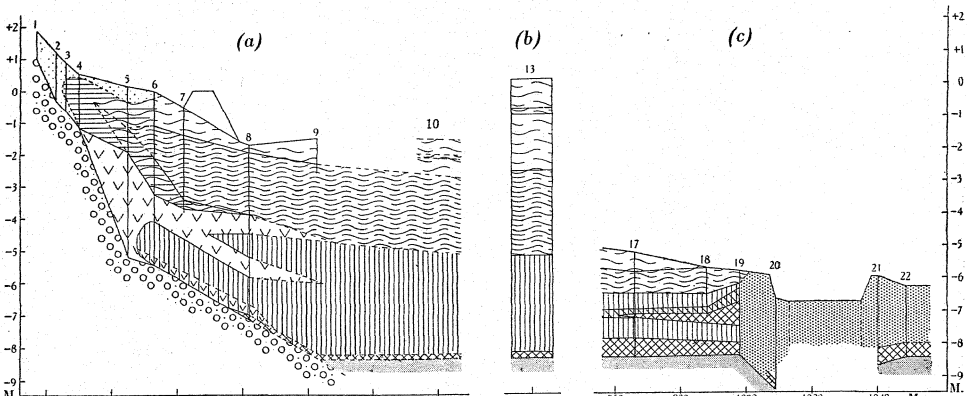
The deposits composing the bog occur in three main facies: (*A*) the central area of the bog (Text-fig. 3 *b*), (*B*) where it abuts against the steeply rising valley side (Text-fig. 3 *a*), and (*C*) where it approaches the Teifi (Text-fig. 3 *c*). In the description which follows, each facies will be described separately, and the deposits will be detailed in order of accumulation, from the bottom upwards.

(A) The central area of the bog is underlain by a blue-grey clay, usually without sand; the clay was very stiff in composition, and the drill could only be forced into it with difficulty. Its surface was horizontal along the line of section, and this fact, coupled with the absence of sand, suggests that it is a lacustrine deposit. The clay lay at a depth of 840 cm. below datum. Resting on this clay was a pale brown mud, without sand, which contained seeds of open-water

[1] The purchase and loan of the drill by the Royal Society of London are gratefully acknowledged.



Text-fig. 2. A. Stratigraphy of the S.E. bog from hill-side to the Teifi. B. Stratigraphy of the W. bog. C. Symbols for peat types employed in this and the other figures.



Text-fig 3. Detailed stratigraphy of the S.E. bog, especially the hill-margin (*a*) and the river-margin (*c*). A typical central profile is shown as (*b*). Symbols as in Text-fig. 2.

plants,[1] as well as scattered fragments of wood and some rootlets. This was succeeded upwards by a *Phragmites* peat, again with scattered pieces of wood. Seeds of *Menyanthes* and *Nuphar luteum* were rather common in the lower layers, and remains of *Cladium* occurred throughout the deposit. It was highly saturated with water, and at times the chamber of the drill failed to open and no sample was obtained.

Highly humified *Sphagnum* peat lay upon the *Phragmites* peat and the transition generally was abrupt; the humification was about 8 on the von Post scale (von Post, 1924). Fibres and roots of *Eriophorum* were fairly plentiful, and scattered tufts of *Scirpus caespitosus* were found, but with the exception of twigs of *Calluna* no wood was present. Here again at various depths it was impossible to obtain a sample. An abrupt transition again separated this peat from the fresh lighter coloured *Sphagnum* peat, of humification about 3, which rested upon it. Remains of *Calluna* and *Eriophorum* were plentiful and *Oxycoccus* was also recorded.

A layer of more highly humified *Sphagnum* peat (H. 7–8), whose thickness varied from 7 to 50 cm., succeeded this fresh peat. The layer could be plainly seen as a rather greasy band in most of the cuttings towards the eastern margin of this central area. Another layer of fresh *Sphagnum* peat (H. 4) formed the uppermost stratum of the central area, and it was clothed by a plant cover formed by *Eriophorum vaginatum* and *angustifolium*, *Calluna*, *Erica tetralix*, *Narthecium ossifragum*, *Scirpus caespitosus*, and *Sphagnum* spp. Signs of extensive burning were obvious, but remains of all these plants could be identified in the upper fresh *Sphagnum* peat.

The sequence of the layers at S.E. 13, in the centre of the bog, gives a typical record for this area (see Text-fig. 3).

0– 90 cm. Fresh light brown *Sphagnum* peat (H. 4), with twigs of *Calluna*, and roots of *Eriophorum vaginatum*.
90–110 cm. Highly humified dark brown *Sphagnum* peat (H. 7–8), with roots of *Eriophorum*.
110–285 cm. Fresh light brown *Sphagnum* peat (H. 4), with twigs of *Calluna*, fibres and roots of *Eriophorum angustifolium*, rootlets of *Oxycoccus*.
285–350 cm. Highly humified dark brown *Sphagnum* peat (H 8), with twigs of *Calluna*, fibres and roots of *Eriophorum*.
350–550 cm. Drill chamber failed to open.

[1] Seeds of aquatic plants found in the light brown mud at the base of the south-east bog include the following: *Cladium mariscus*, *Comarum palustres*, *Menyanthes trifoliata*, *Myriophyllum*, *Naias flexilis*, *Nuphar luteum*, *Nymphaea alba*, *Potamogeton* cf. *pusillus*, *natans* et spp., *Ranunculus aquatilis*, *Scirpus lacustris*, *Sparganium* sp., and *Viola palustris*. *Chara* oospores were also found.

550–850 cm. Highly humified peat, with fibres and roots of *Phragmites* and *Cladium*. Scattered pieces of wood, mainly *Betula*.
850–870 cm. Brown mud, scattered rootlets. Seeds of *Cladium mariscus*, *Scirpus lacustris*, *Potamogeton natans*, *alpinus* (?) spp., *Nuphar luteum*, *Naias flexilis*, *Comarum palustre*, *Betula* sp., also *Chara* oospore, *Pediastrum* spp., sponge spicules, *Cladocera*, diatoms, twigs of *Betula*.
870–875 cm. Stiff blue-grey clay.

(B) The stratification of the eastern end of the bog, where it abutted against the hillside, was more complicated (Text-fig. 3*a*). The borings which were highest on the slope revealed a greyish silt mixed with plant debris (roots, fibre and wood), and in S.E. 4 170 cm. of yellow-grey peat with a high silt content and some wood debris were traversed before the drill reached stone.

The next bore S.E. 5, however, penetrated to a depth of 533 cm. (520 cm. below datum), at which depth an angular gravelly hillwash was encountered.

0– 30 cm. Sand-free silt.
30– 50 cm. Dark brown silt, with plant debris, including pieces of wood.
50–130 cm. Yellow-brown peat, with many yellow fibres and roots (*Molinia* ?). Traces of wood debris.
130–220 cm. As above, but containing several brown layers rich in wood debris.
220–505 cm. Red-brown amorphous peat, composed of wood debris. *Cenococcum* very frequent. Between 400 and 500 cm. many large pieces of wood.
505–525 cm. As above, but with some layers of grey silt, latter becoming more numerous below.
525–528 cm. Sand-free grey-white silt.
528–533 cm. Brown amorphous peat.
533–535 cm. Sandy silt with pebbles. Drill among stones.

As the record shows, about 3 m. of brushwood peat rest on the hill-wash, and this is succeeded in turn by a yellow-brown peat which contains brown layers with much wood debris. No *Calluna* or *Eriophorum* could be seen in this peat, nor were they present in the yellow-brown peat without much wood content, which lay upon it. It is probable that the yellow fibres and roots which formed the bulk of this peat were derived from *Molinia*.

S.E. 6, only 8 m. away from S.E. 5, shows very clearly the inter-relations of the vegetation of the hill-slope with that of the bog proper.

0– 25 cm. Disturbed.
25–110 cm. Brown highly humified *Sphagnum* peat (H. 7) with twigs of *Calluna*, and fibres and roots of *Eriophorum*. From 50 to 60 cm. layer with some content of *Molinia*.

110–210 cm. Red-brown highly humified peat (H. 8), with varying content of wood debris, mainly *Betula*. Twigs of *Calluna* and fibres of *Eriophorum*.
210–250 cm. Yellow-brown peat with yellow fibres and traces of *Eriophorum* fibre. Much wood debris, mainly *Betula*.
250–290 cm. Red-brown peat with scattered twigs of *Calluna*.
290–325 cm. Yellow-brown peat, with much yellow fibre. Traces of *Eriophorum* and scattered fragments of wood.
325–410 cm. Red-brown amorphous peat, composed of wood debris with *Cenococcum*.
410–450 cm. Brown peat with much wood debris, but also roots and fibres of *Phragmites*.
450–540 cm. As above, but with *Phragmites* content decreasing downwards and wood content increasing downwards, Hazel nut (*Corylus*) at 535 cm.
540–545 cm. Brown-black amorphous peat, without sand.
545–550 cm. Stiff grey clay, with small stones.

Here the gravel lies at a depth of 545 cm. (545 cm. below datum), and a peat mainly composed of wood debris, but with some content of *Phragmites*, rests on it. The amount of *Phragmites* increases in the upper layers. A considerable layer of amorphous brushwood caps this, and then in turn gives way to a yellow-brown peat with some wood debris and also traces of *Eriophorum*; in the middle of this peat a layer redder in colour, and with scattered twigs of *Calluna* occurs. In the next stratum, the presence of *Calluna* twigs and *Eriophorum* fibres, together with a red-brown colour and a high degree of humification (H. 8), suggests that it is a *Sphagnum* peat. It contains a considerable amount of wood debris, in contrast to the topmost layer which is free from wood remains. This upper layer is also more brown in colour and slightly less humified (H. 7); remains of *Calluna* and *Eriophorum* were present. As well, at a depth of 55 cm., *Molinia* fibres were recognizable.

In S.E. 8 the importance of the marginal elements was considerably lessened.

Upper surface of bog removed by cutting.

0– 23 cm. Dark brown fresh *Sphagnum* peat, rather dry.
23–220 cm. Dark brown highly humified *Sphagnum* peat (H. 6–7), with scattered twigs of *Calluna*, much fibre and roots of *Eriophorum*, mainly *vaginatum*, traces of *Oxycoccus*.
220–280 cm. Dark brown peat, very rich in wood debris (mainly *Betula*), no trace of *Eriophorum* or *Calluna*.
280–310 cm. As above, but with some content of *Phragmites*.
310–350 cm. Yellow-brown peat with many fibres and roots of *Phragmites*, traces of wood, cf. *Salix*.
350–415 cm. Dark brown peat, rich in wood debris. Between 370 and 400 cm., chamber blocked by a vertical piece of wood, cf. *Salix*.
415–450 cm. Yellow-brown peat, with many fibres and roots of *Phragmites*, traces of wood.

450–530 cm. As above, but redder in colour, and with greater content of wood debris.
530–545 cm. Grey-brown mud, with some fibre.
545–560 cm. Grey silty sand.

A grey silty sand forms the basal layer here, at a depth of 715 cm. below datum, and on this rests 15 cm. of a light brown mud, comparable with that seen in the central area. This is succeeded by a *Phragmites* peat of which the base is rich in fragments of wood. Next comes a stratum of brushwood peat, then *Phragmites* peat with scattered wood debris in its lower layers, but with wood content increasing upwards till the *Phragmites* disappears and a brushwood peat is established once more. An abrupt transition separates this brushwood peat from its successor, a dark brown well-humified *Sphagnum* peat, with fibres and roots of *Eriophorum* and twigs of *Calluna*, but no other wood remains. On this rests but 23 cm. of fresh *Sphagnum* peat, all the upper layers having been removed by cutting.

The next boring S.E. 10, made at a distance of 100 m. to the west, revealed the stratification typical of the central area of the bog.

(C) Where the bog approaches the Teifi extensive cutting of the upper layers had taken place, and the thin humified layer occurring in the upper fresh *Sphagnum* peat was truncated about 200 m. from the river margin. Here the stratification of the bog belonged to the regime of the central area. At S.E. 16, however, a difference appears (see Text-fig. 3 *c*), as the *Phragmites* peat includes a layer with some mud content, and at S.E. 17 the stratification was as follows:

0– 60 cm. Light brown fresh *Sphagnum* peat (H. 3).
60–125 cm. Brown *Sphagnum* peat (H. 7–8), with scattered twigs of *Calluna*.
125–190 cm. Dark brown *Phragmites* peat, with some mud content in the lower layers.
190–260 cm. Brown *Phragmites* peat, with some *Cladium*, fragments of wood (cf. *Salix*).
260–320 cm. Light brown mud, with some silt content.
320–325 cm. Blue-grey clay.

The basal clay again lay at 840 cm. below datum, and on it was resting 60 cm. of brown mud. Capping this was a brown *Phragmites* peat, and then another thin layer of light brown mud. This passed upwards into a muddy *Phragmites* peat of a dark brown colour, in whose upper layers the mud content was small. Then the *Sphagnum* peats appear, highly humified below, and only slightly humified above.

S.E. 18 ended in the upper mud and in reaching this the following layers were encountered:

0– 80 cm.	Dark brown highly humified *Sphagnum* peat, with some content of *Eriophorum*.
80–120 cm.	Brown fibrous peat, containing some *Scirpus* and a few wood fragments.
120–145 cm.	Black muddy peat, with some content of *Phragmites* and traces of wood and bark, mainly *Betula*.
145–150 cm.	Brown mud peat rich in wood debris.

The record of S.E. 19 was:

0– 25 cm.	Disturbed.
25– 40 cm.	Brown highly humified peat, traces of *Eriophorum*.
40– 55 cm.	Dark brown peat with some yellow fibre.
55– 95 cm.	Brown fibrous peat, with some content of mud.
95–182 cm.	Brown peaty mud, with scattered fibres of *Phragmites*. Also traces of *Betula* bark and wood. At *c.* 110 cm. some slight content of grey silt.
182–220 cm.	Black-brown peat, rich in fibres and roots of *Phragmites*. Seed of *Potamogeton natans*.
220–250 cm.	Brown mud, becoming lighter in colour below. Many seeds of *Potamogeton* spp.
250–262 cm.	Green-brown mud.
262–267 cm.	Grey clay.

The grey clay was once again at 840 cm. below datum, and above this all the layers seen at S.E. 17 were present, with the exception of the fresh *Sphagnum* peat: a highly humified peat, containing *Eriophorum*, which was in all probability a *Sphagnum* peat, was the uppermost layer. The upper mud layer was considerably thicker here, and contained traces of silt.

The boring S.E. 20 at the eastern margin of the river showed a different sequence. The clay was reached at 945 cm. below datum: on it lay 50 cm. of mud and then 250 cm. of sandy silt with some vegetable content, including remains of *Phragmites*. A slightly elevated bank (perhaps artificial) formed the western margin of the stream, and then a rather level plain stretched away to meet the cut-away southern margin of the western bog. Under this plain near the river, the blue-grey clay lay at a depth of 840 cm. below datum and was covered first by a layer of mud, and then by silt. A boring S.E. 21 in the elevated bank reached the clay at a depth of 860 cm. below datum, having passed through silt and mud.

It is unlikely that the river could have cut this channel in the blue-grey clay (seen in S.E. 20 and 21) until its flow had been constricted by the formation of peat on either side. Therefore it is probable that the mud lining the channel is the equivalent of the

upper mud in the margin of the bog proper, but as the connexion was not established in the field, these two mud layers have not been linked up in the figure (Text-fig. 3*c*).

The plain referred to above was apparently a flood-plain, and lay in the angle between the Teifi and its tributary, the Cambddwr. A flood-plain also stretched from below this union with the Cambddwr, on the western side of the Teifi, to where the river crossed the moraine at Pont Einon. The level of the plain at the bridge was 670 cm. below datum, or 50 cm. below the level of the plain at the western end of the section.

The cross-section of the bog (seen in Text-fig. 2A) shows clearly the domed structure of the central area. The dome slopes away rather evenly to either side, and towards the river there is a fall of about 650 cm. before the level of the flood-plain is reached. Near the hill-side the natural slope of the bog is interfered with by the railway embankment, but in all probability there was a fall on this side of about 250 cm. and then the bog rose again on to the marginal hill-slopes, where the highest peat occurs at about the same level as the summit of the dome.

III. STRATIGRAPHY OF THE WEST BOG

The line of section in the western bog was not so continuous, and is best considered as being in two loosely connected parts (Text-fig. 2B). The line began at a place on the western side of the Teifi, where the margin of the bog was intact, about 1000 m. upstream from the section in the south-eastern bog (Fig. 1). From the river it ran to the centre in a north-westerly direction, and at right-angles to the river. The divide separating the basins of the Teifi and the Cambddwr ends in a peninsula of high ground, which runs out in a south-westerly direction into the bog, and is almost surrounded by it. Against the ridge the raised bog proper ends. In some cuttings at the margin, trunks of *Quercus* were seen; a short section ran out at right-angles from the margin a little way into the bog, and approximately north-north-west to south-south-east. The end of this section was linked to the centre of the bog by a line running north to south; only two borings were made on this segment. The total length of the line was about 1500 m., and though fifteen borings were made, many more would be necessary to reveal the full detail of the stratigraphy. Serial numbers in succession to those in the other bog were given to the borings from east to west, with the letter W.

prefixed. Unfortunately the bench-marks of the Ordnance Survey nearest to the bog could not be discovered, and so the datum, established for the south-eastern bog, was carried forward upstream to the river end of this bog.

The deposits composing the bog will be described as (A) from the Teifi to the centre of the bog (Text-figs. 4*a*, *b*), (B) the north-west margin (Text-fig. 4*c*), and (C) the segment connecting the two preceding sections.

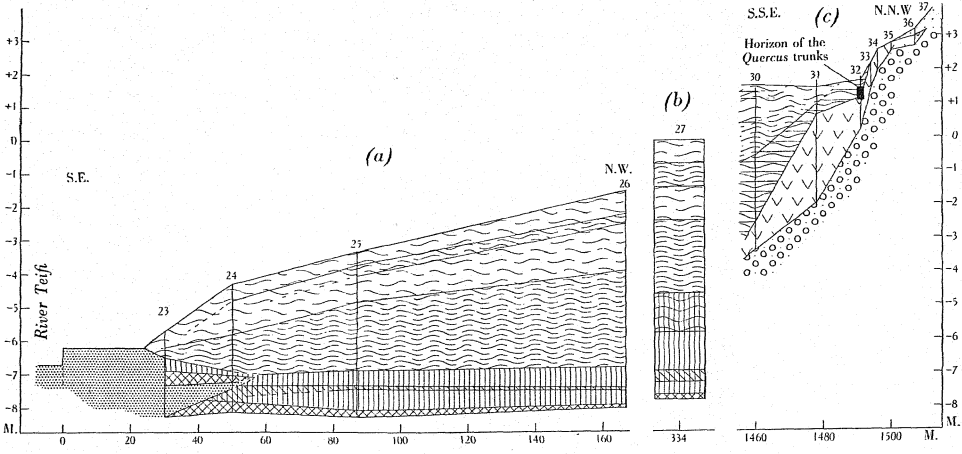
(A) The record of W. 27, where by coincidence the surface of the bog was at the same level as datum, gives a typical picture of the sequence in the central area:

0– 70 cm. Brown *Sphagnum* peat (H. 4–5).
70–140 cm. Brown *Sphagnum* peat (H. 7).
140–240 cm. Red-brown *Sphagnum* peat (H. 3), with scattered twigs of *Calluna*, some fibre of *Eriophorum*, rootlets of *Oxycoccus*.
240–460 cm. Brown *Sphagnum* peat (H. 8), with scattered twigs of *Calluna*, some fibre of *Eriophorum*, rootlets of *Oxycoccus*.
460–575 cm. As above, but darker in colour and with some yellow fibres. In lower layers some content of *Phragmites*.
575–690 cm. Brown *Phragmites* peat, with scattered pieces of wood, and seeds of *Menyanthes* in lower layers.
690–725 cm. Grey-brown mud peat, with some *Phragmites* and scattered pieces of wood.
725–760 cm. Grey-brown *Phragmites* peat, with seeds of *Menyanthes* and scattered pieces of wood.
760–775 cm. Brown mud peat, with some content of *Phragmites*. Seed of *Nuphar luteum*, seed of *Carex* sp.
775–785 cm. Grey clay, top 3–4 cm. rather sandy.

Except near the hillside margin, blue-grey clay occurred at the base of this bog also, and at W. 27 it lay at a depth of 775 cm. below datum. At W. 28, which was regarded as the centre of the bog, and the end of this part of the line, the blue clay lay at a depth of 7 m. below datum, and resting on it were 860 cm. of bog deposits.

It is obvious that in this area the stratification is very similar to that in the centre of the south-eastern bog. Towards the river the slope of the bog surface steepens, and the slight fall in level of the blue clay continues. At W. 25, about 90 m. from the river, the clay is 820 cm. below datum, and though the bog is only 490 cm. deep, the layers are essentially those of the centre of the bog. But in W. 24 a new facies appears in the lower layers, and the *Sphagnum* peat is thinner:

0– 50 cm. Brown *Sphagnum* peat (H. 5), twigs of *Calluna*.
50–150 cm. Red-brown peat (H. 7), twigs of *Calluna* and scattered fibres of *Eriophorum*.
150–257 cm. Brown peat (H. 9), twigs of *Calluna* and varying content of fibres of *Eriophorum*.



Text-fig. 4. Detailed stratigraphy of the W. bog, especially the river margin (*a*) and the hill margin (*c*). A typical central profile is shown as (*b*). Symbols as in Text-fig. 2.

257–270 cm. Black-brown peat, with some yellow fibre and remains of *Carex* (?).
270–283 cm. Brown mud peat.
283–290 cm. Chamber filled with wood.
290–307 cm. Grey-brown silt, with some vegetable content.
307–350 cm. Brown mud peat, with traces of *Phragmites* and scattered pieces of wood. Seed of *Nuphar*, some leaf fragments.
350–383 cm. Brown mud with scattered pieces of wood, also some seeds of *Scirpus lacustris*, *Potamogeton natans* spp.
383–400 cm. Blue-grey clay, top 5 cm. rather sandy.

Here, as before, the basal clay (at a depth of 815 cm. below datum) is capped by a layer of mud, though the thickness of the latter is increased. Then comes a layer of silt and then mud again which passes gradually up into the *Sphagnum* peats. W. 23 shows the silt again, and here no layer of mud separates it from the basal clay:

0– 50 cm. Dark-brown peat (H. 5), with scattered twigs of *Calluna* and fibres of *Eriophorum*.
50– 80 cm. As above, but more highly humified (H. 8).
80–120 cm. Dark brown amorphous peat (H. 8), with fragments of bark and twigs of *Betula*.
120–158 cm. Brown crumbling mud peat, rich in wood debris, mainly *Betula*. Traces of *Phragmites* in lower layers.
158–255 cm. Alternating layers of grey silt and brown peat rich in wood debris.
255–260 cm. Blue-grey clay.

Above the silt, the sequence is much as in W. 24, and below it the clay lies 825 cm. below datum.

The absence of silt in the *Phragmites* peat and mud, against which the silt abuts, suggests that the silt is later than the peat and mud, and not contemporaneous with them. A renewal of river activity, and migration of its channel, followed by quieter conditions with deposition of silt and mud, are clearly indicated. This mud was then overgrown, and conditions favourable for the growth of *Sphagnum* peat established. It should be noted that this mud is at approximately the same level as the upper mud in the south-eastern bog, and that there also the upper mud is rich in wood debris.

(B) The section at the north-western margin of the bog is about 50 m. long and depends in the main on three borings. On the hill slope, about 300 cm. above datum, a thin layer of peat, rich in wood debris, occurs under a thin layer of silt, washed down from the slope above. As the peat is traced down the slope, the silt disappears, and at W. 32 the following sequence was recorded:

0–13 cm. Disturbed.
13–35 cm. Brown peat, rich in wood debris, mainly *Betula*.
35–60 cm. Light brown peat, with much fibre of *Molinia*, and some wood debris.

60– 65 cm. Fibrous peat, formed mainly of *Polytrichum* stems.
65–100 cm. Brown peat, rich in wood. Two metres north of the profile, a large *Quercus* trunk, 50 cm. in diameter, was lying horizontally, with its upper surface 30 cm. below the surface of the ground. Several other *Quercus* trunks of similar dimensions were to be seen lying horizontally in a trench which ran along the margin of the bog at this point.
100–150 cm. Yellow-brown peat, with many fibres of *Molinia*, and rich in wood debris.
150–160 cm. Brown peat, rich in wood debris.
160–185 cm. Brown silt, with sand and small pebbles. Some traces of vegetable debris.
185–200 cm. Grey silty gravel, with pebbles *c.* 2 cm. in diameter.

The basal peat was here 25 cm. above datum, and was a brushwood peat, with some *Molinia* in its lower layers. In the upper layers the *Quercus* trunks were found, and they appeared to have fallen in from the marginal slope towards the bog. On top of this brushwood peat, another peat, rich in *Molinia*, occurred.

Sphagnum peat appears in W. 31:

0– 20 cm. Disturbed.
20– 70 cm. Dark brown highly humified *Sphagnum* peat, with remains of *Calluna* and *Eriophorum*, and scattered pieces of wood.
70– 83 cm. Yellow-brown fibrous peat.
83–100 cm. Red-brown highly humified *Sphagnum* peat, with *Calluna* and *Eriophorum*.
100–365 cm. Amorphous brown brushwood peat, with many pieces of wood, mainly *Betula*.
365–400 cm. Light grey-brown sandy clay, with small pebbles.

and in W. 30 all the upper layers of the central area are present, though the sequence ends in brushwood peat at a depth of 335 cm. below datum:

0– 18 cm. Fresh rather dry *Sphagnum* peat.
18– 35 cm. Dark brown humified *Sphagnum* peat (H. 6).
35–110 cm. Red-brown fresh *Sphagnum* peat (H. 3), with scattered twigs of *Calluna*.
110–210 cm. Brown highly humified *Sphagnum* peat (H 8), with twigs of *Calluna* and varying content of *Eriophorum* fibre.
210–260 cm. As above, but with some yellow fibres (cf. *Molinia*).
260–270 cm. Red-brown peat, rich in moss stems.
270–315 cm. As above, but with yellow fibres.
315–370 cm. Red-brown highly humified peat, without fibre, but with scattered twigs of *Calluna*.
370–410 cm. Yellow-brown peat, rich in fibres of *Molinia*, with scattered wood debris.
410–495 cm. Red-brown amorphous brushwood peat.
495–530 cm. Grey sandy silt, with scattered vegetable debris.
530–550 cm. Blue clay with pebbles.

(C) In the segment of the line that connected the ends of the two preceding sections, it was very difficult to make satisfactory

borings, due to the saturation of the intermediate layers with water. At W. 29 a layer of *Phragmites* peat with wood debris, 130 cm. thick, rested on the clay at a depth of 740 cm. below datum, and above this peat all the *Sphagnum* layers were represented. The greatest depth of bog deposits was recorded here, a total thickness of 940 cm. Between this point and W. 28, where the section from the river ended, the bog reached its greatest elevation 220 cm. over datum.

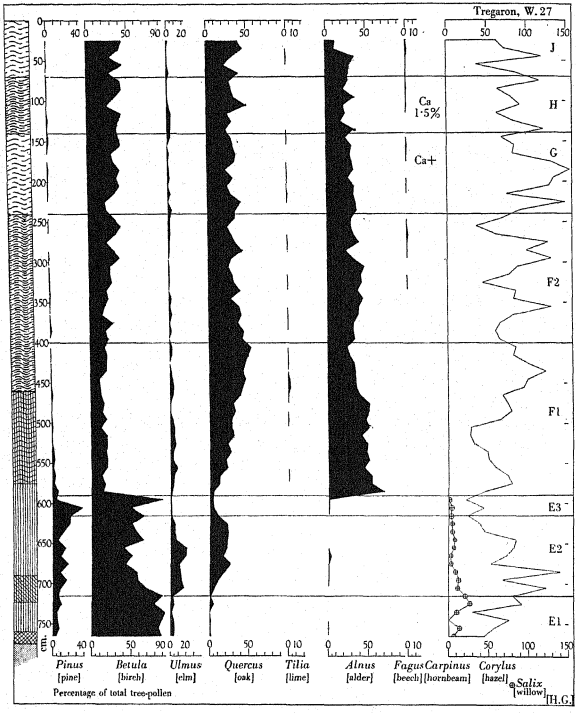
Because the section in this bog was not consistent in direction, it has not been drawn as a continuous whole (see Text-fig. 2 *b*). Some portions of little interest have also been omitted. The slope of the bog from its raised centre to the margin of the Teifi is clearly seen; the fall in height is about 800 cm. At the northern end of the section, the bog meets the lower slopes of a peninsula of high ground. The bog surface is here almost horizontal, being only 60 cm. lower in level than the highest part of the bog and a lagg is only feebly developed.

IV. Results of pollen analysis

Pollen diagrams are available from four borings, one in the western bog at W. 27, and the other three in the south-eastern bog, S.E. 6 close to the hillside margin, S.E. 10, rather nearer to the centre of the bog and S.E. 18 at the river margin.

Diagram W. 27 (Text-fig. 5). It is convenient to consider this diagram first, for, being derived from samples taken in the centre of the valley, in it local influences should be minimal, and the pollen curves should most closely represent the forest history of the countryside as a whole. It will be observed at once that the diagram reflects two sharply differing phases of forest history. That below 590 cm., or zone E, is characterized by dominance of *Betula*, with smaller amounts of *Pinus*. *Ulmus* and *Quercus* are present throughout, in fair amounts in the middle of the phase, where also a little *Alnus* is present: *Tilia*, *Fagus* and *Carpinus* are absent, but *Corylus*, present from the beginning, reaches high values in the middle of the period. Above 590 cm. woodland dominance was apparently shared by *Alnus*, *Quercus* and *Betula*, the transition from zone E being marked by the abrupt rise of the *Alnus* curve which suddenly displaces *Betula* and *Pinus* from dominance. Not far above this horizon small quantities of *Tilia* pollen begin to appear in the samples. The diagram, above 590 cm., shows no further sudden changes in pollen composition, and the steady drift of all the pollen curves is, in fact,

extremely striking. It should be noted that *Quercus* has a maximum about 400 cm., that *Betula* rises steadily in value as the upper



Text-fig. 5. Pollen-diagram at W. 27 (W. bog). Symbols as in Text-fig. 2.

layers are reached, that above 150 cm. *Tilia* is almost absent, and that above 340 cm. *Fagus* and traces of *Carpinus* are present. The *Corylus* curve shows a general drift from low values at the end of E

to high values about 200 cm. and then a decline, but on this drift is superimposed a shorter rhythm of rise and fall, at present not explained. In view of the absence of comparable diagrams from other British deposits, the authors do not at present feel it advisable to use these data for zoning the younger peat, and prefer instead to employ the stratigraphic criteria which are well-marked in all three bogs in the Teifi valley.

The zones so marked are:

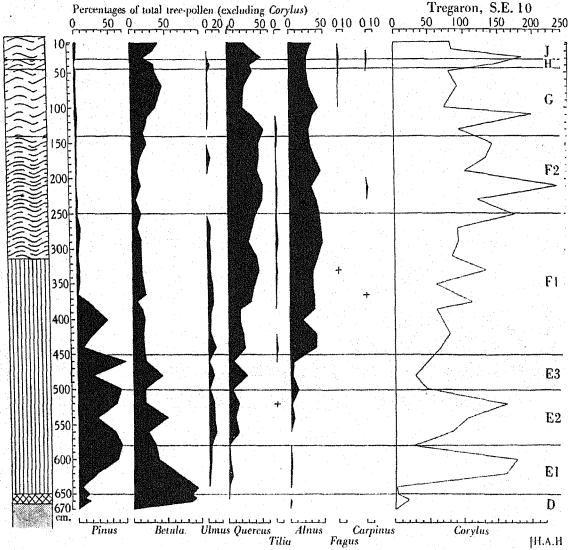
F ... From 590 cm., to the marked decrease in humification at 235 cm.
G ... The fresh *Sphagnum* peat between 235 and 135 cm.
H ... The highly humified *Sphagnum* peat between 135 and 60 cm.
J ... The fresh *Sphagnum* peat above 60 cm.

Within zone E it is possible to recognize three subzones. The earliest, E 1, shows low values for *Ulmus* and *Quercus*, while *Betula* is dominant: the middle subzone, E 2, has substantial amounts of *Ulmus* and *Quercus*, together with a little *Alnus*: at the onset of E 2 *Ulmus* exceeds *Quercus*, and there is a high *Corylus* maximum; other *Corylus* maxima are well marked in this zone. The latest, E 3, shows a recession of *Ulmus* and *Quercus* with low *Corylus* values, and a re-advance of *Pinus* and *Betula*. It is also possible to split F into two subzones F 1 and F 2, one before and one after the *Quercus* maximum.

Diagram S.E. 10 (Text-fig. 6). The profile illustrated by this diagram is again one which extends through the full extent of the bog deposits from the basal lake mud to the living surface. Here all the zones into which W. 27 was divided are recognizable, but in addition, at the base it is necessary to add an earlier zone D. This zone, from 670 to 650 cm., is marked by extreme dominance of *Betula* and complete absence of *Quercus* and *Ulmus*: *Corylus* pollen also is extremely sparse in comparison with the huge values which it attains in E.

Zone E ends at 450 cm., where *Alnus* rises suddenly and *Tilia* effectively begins. E 1 extends from 650 to 580 cm., where, in E 2, *Ulmus* and *Quercus* rise, and *Alnus* shows in small quantity as in diagram W. 27. It is striking that here *Corylus* shows a marked maximum; this may relate to the smaller peak at 735 cm. in diagram W. 27. E 3 is not readily separable from E 2, though low *Corylus* values give some grounds for making the distinction. It is interesting to note, in comparison with the diagram from the western bog, how in this bog much *Pinus* is present in proportion to *Betula*, though the relative amounts of the other pollens are not significantly different. It is unlikely that the effect can be due to *Pinus* on the bog surface

close by, for at this time fen peat with *Phragmites* seems to have been prevalent. It is possible that local stands of *Pinus* on the outwash gravels down the valley contributed more to the nearer diagram than to the other. The effect is maintained into the early part of F 1.

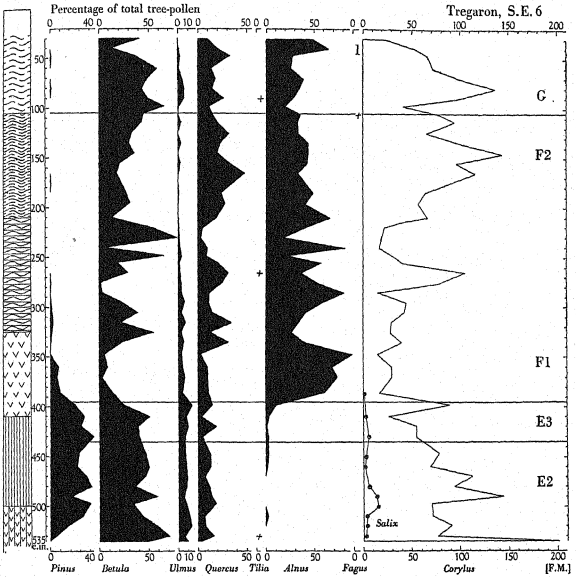


Text-fig. 6. Pollen-diagram at S.E. 10 (S.E. bog). Symbols as in Text-fig. 2.

Zone F is much as in the diagram from the other bog, but it should be noted as well that in F 1 both *Pinus* and *Ulmus* have higher values than in F 2. *Tilia* is present in small amounts throughout, and a few scattered grains of *Fagus* and *Carpinus* occur. Zone G, above the marked lessening in humification, is marked by the disappearance of *Tilia* and the appearance of small but continuous amounts of *Fagus*. *Betula* here shows a much sharper rise than in

diagram A. The upper layer of highly humified peat is not sharply set off, and zones H and J have no strong characterization in the pollen curves.

DIAGRAM S.E. 6 (Text-fig. 7). Like the two diagrams already considered, this diagram shows a very evident division into two

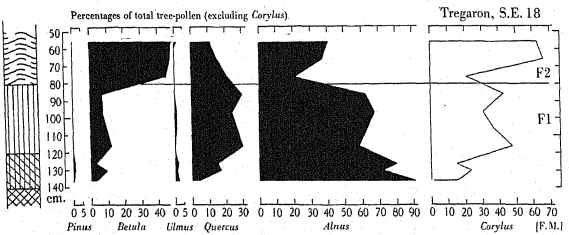


Text-fig. 7. Pollen-diagram at S.E. 6 (S.E. bog). Symbols as in Text-fig. 2.

halves, only the upper of which contains much *Alnus*. The transition, at 395 cm., separates phases E and F. It is clear that the base lies in E 2, for values of *Ulmus* and *Quercus* are substantial, *Corylus* is abundant, and *Betula* is diminishing. E 3 is not sharply separable from E 2, but it may be regarded as extending from 435 to 395 cm., on the grounds of lowered *Quercus* and *Corylus*, and high values for

Pinus and then *Betula*. Throughout E 2 and E 3, this site shows values for *Pinus* almost as high as in S.E. 10 and these are probably attributable to the same cause. *Pinus* values of about 10% persist here also into zone F.

This site is marginal, and so lacks the stratigraphical indices available in the other diagrams. It seems probable, however, that the boundary between F and G should be placed at 110 cm., where a *Sphagnum* peat without wood first extends over a *Sphagnum* peat rich in wood. Such a division has stratigraphical conformity with the central area, where a marked change is obvious in the *Sphagnum* peat, and is itself indicative of a renewal of growth in the bog proper. If zone F is therefore considered to extend from 395 to 110 cm., it clearly includes marked changes in the development of peat in the



Text-fig. 8. Pollen-diagram at S.E. 18 (S.E. bog). Symbols as in Text-fig. 2.

area, and may moreover be readily divided into an earlier and a later part. The earlier part, F 1, shows the slow disappearance of *Pinus* and *Ulmus*; it has begun with a wood peat doubtless dominated by *Alnus*, and it ends, from 250 to 210 cm., in a phase clearly dominated by local influences of *Alnus* and *Betula*. Pollen from these trees lowers the values of such trees as *Quercus* and *Corylus*, which could not have grown on the bog surface. The later part, F 2, shows no *Pinus* and little *Ulmus*, but exhibits a rise in *Corylus* to high peak values broadly like those in the same subzone in diagrams W. 27 and S.E. 10. Above 110 cm. (where zone G begins) it is not feasible to zone the diagram, but it should be noted that immediately above this level, presumably still in zone G, a well-marked secondary maximum of *Ulmus* occurs. The small amounts of *Fagus* pollen found at this site all occur above 120 cm.

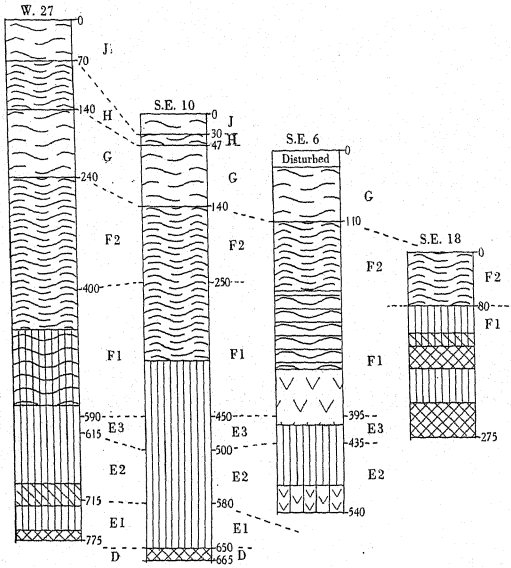
DIAGRAM S.E. 18 (Text-fig. 8). The pollen profile at this boring is

quite shallow, extending only from 55 to 135 cm. Nevertheless, it crosses three distinct layers recognizable in the general stratigraphy, *Sphagnum* peat, fen peat and muddy *Phragmites* peat. The high *Alnus* values indicate that even the base of the diagram is later than zone E, and the very low *Ulmus* values and the effective disappearance of *Pinus* suggest that the lowest samples are near the end of F 1. This is strongly supported by the low *Corylus* values, though *Corylus* rises in the upper samples. This rise suggests that the boundary between F 1 and F 2 should be placed at about 80 cm. In connexion with this it is worth noting that this is the horizon at which a marked alteration occurs in the *Alnus-Betula* ratio. Though it is difficult to attribute the change to anything but local factors, it agrees well with the similar development at the F 1/F 2 boundary in diagram S.E. 6.

V. Discussion (*a*) of the development of the bogs as indicated by the stratigraphy and pollen analyses, and (*b*) of the regional significance of the pollen diagrams

(*a*) (See Text-fig. 9.) The final retreat of ice from this area left the valley of the Teifi blocked by a bar of moraine, below which extensive outwash gravels stretched down the valley for several miles. Behind this bar the river water was impounded to form a lake of considerable size, and on the floor of the lake a blue-grey clay, with some content of sand but without plant remains, was deposited. This clay is probably late glacial in age, and was laid down before the plant cover had been restored on the surrounding countryside. On an amelioration of the climate, reclothing of the area reduced the amount of clay and sand available for river transport, and the clay was succeeded by an organic mud, light brown in colour. The nature of the mud, and the seeds which it contains, make it obvious that this is an open-water deposit, a true nekron-mud. The presence of seeds of *Naias flexilis* (identification kindly confirmed by Prof. K. Jessen, Copenhagen) in this mud of early post-glacial age is of considerable interest. Common in North America, this plant has now a discontinuous and markedly "Atlantic" distribution in Europe; Kerry, west Galway and west Donegal in Ireland, Perth and Skye in Scotland, Esthwaite Water in Lancashire and a few stations in north-west Europe (Praeger, 1934). But in Scandinavia and Finland seeds of this plant have been found in about fifty localities, in mud

principally of Boreal age (Jessen, 1934). Jessen has also recorded *Naias flexilis* in a *Phragmites-Cladium* peat of late Boreal age in the Bann Valley, in north-east Ireland (Jessen in Movius, 1936).



Text-fig. 9. Correlation figure showing presumed relation between the stratigraphy of the four sites examined by pollen-analysis. The first three have been aligned on the transition from E 3 to F 1.

This mud is not found higher than 675 cm. below datum, but it is present at the bases of diagrams W. 27 and S.E. 10. At S.E. 10, where the level is slightly lower than at W. 27, deposition took place in zone D, and the mud is replaced by a *Phragmites* peat before zone E commences. At W. 27 zone D is not represented, but the deposition of mud had ceased before subzone E 1 came to

an end. So that by the end of E 1, the open water had been largely obliterated by the development of a *Phragmites* community, which grew out over the lake floor, and through which the Teifi made its sluggish way. A brushwood peat, with some slight content of *Phragmites*, forms the base of diagram S.E. 6, which lies in subzone E 2. The content of *Phragmites* in this peat increases upwards, and this may indicate a rise of the water level in the basin, the *Phragmites* association pushing up the hillside among the trees, and overwhelming those which stood on the lower slopes. In this connexion it should be noted that an extensive horizon of mud occurs in the *Phragmites* peat below the western bog, and in diagram W. 27 this mud lies in the lower part of subzone E 2. The subzone E 3 is marked by a *Pinus* maximum, and this is more prominent in the diagrams from the south-eastern bog than in the diagram from the western bog. It has been already suggested that this is a local feature, being possibly caused by a stand of *Pinus* on the outwash gravels to the south of the moraine. In both diagrams S.E. 6 and S.E. 10, this *Pinus* maximum extends, though in a reduced amount, into the next zone F.

The abrupt rise in the importance of *Alnus* as a forest component marks the beginning of zone F, though pollen of this tree was present in small quantities in the later stages of zone E. At S.E. 6 the commencement of zone F is marked stratigraphically by an extension of the trees out over the *Phragmites* margin. Doubtless *Alnus* was an important member of this brushwood. Not at S.E. 6, but at S.E. 8, this brushwood peat is divided into two by a layer of *Phragmites* peat. The lower brushwood peat is succeeded by a true *Phragmites* peat, which with increasing wood content grades gradually into the upper brushwood peat. It is suggested that the single horizon of brushwood peat at S.E. 6 is the equivalent of these three layers at S.E. 8.

This reappearance of *Phragmites* peat suggests again a rise in water level, and this is supported by the stratigraphy near the Teifi. Here a layer of open-water mud appears in the *Phragmites* peat, which occurs above and below it. A muddy stratum in the *Phragmites* peat carries the mud horizon a long way into the bog. Unfortunately the pollen samples of diagram S.E. 18 end 5 cm. above the mud and do not reach it, but the lower samples fall in subzone F 1, and the age of the *Phragmites* peat, still muddy at this level, is the same as that of the upper centimetres of the brushwood peat of diagram S.E. 6. If the suggestion of the preceding paragraph is accepted,

then the overgrowth of the mud near the river by *Phragmites* is approximately contemporaneous with the re-advance of the trees over the marginal *Phragmites* at the other side of the bog.

It will be noted (see Fig. 3*c*) that a channel is cut in the basal clay underneath the present course of the Teifi along the western margin of the south-east bog. This channel is now filled, first by a layer of mud, and then by silt. It is unlikely that this groove could have been cut before the course of the river had been constricted by the growth of *Phragmites* on either side of it. A similar feature is seen beneath the river-side margin of the western bog. Here, after the deposition of successive layers of mud, *Phragmites* peat, mud and then *Phragmites* peat again which took place throughout zone E, the river cut a channel through these earlier deposits to the basal clay, subsequently filling this channel with alternating layers of silt and mud-peat. These layers are succeeded by an open-water mud which passes upwards into a fen peat.

Thus beneath the western bog there is evidence of increased activity on the part of the Teifi after zone E had formed, followed by calmer open water with the deposition of mud, and at the river margin of the south-eastern bog there is evidence of increased activity by the Teifi, and evidence of open water with deposition of mud during the early part of zone F. It is not unlikely that all these events are interrelated, first increased flow of the river, followed by ponding and rise in water level, with deposition of mud near the river and advance of *Phragmites* over the marginal brushwood, and then disappearance of the open water and a re-advance of the brushwood. These events took place in F 1.

The mud-phase in the *Phragmites* peat of the west bog, shewn at W. 27, must have been in E 1 or E 2, that is far earlier than the cutting of the Teifi channel and the deposition of mud which followed it, and which presumably caused the mud phase in the *Phragmites* peat of the south-east bog during F 1.

The next phase is the appearance of the plants characteristic of the raised bog proper. This change over seems to have taken place first in the western bog, at an early stage in F 1. The transition here is not abrupt, and suggests a gradual displacement of the *Phragmites* community. The appearance of *Sphagnum* spores and grains of ericoid pollen in the upper layers of the true *Phragmites* peat in diagram W. 27 makes it certain that the first appearance of this community was not at this point, but elsewhere in the valley. At a later stage in F 1 *Sphagnum* makes an abrupt entry in diagram

S.E. 10, and at about the same time the brushwood peat in diagram S.E. 6 gives place to a pale-coloured fibrous peat, containing remains of *Sphagnum* and *Molinia*. The lower layers of this peat do contain some layers rich in wood, but above this level brushwood peat does not occur again.

This change also took place at the hillside margin of the western bog, where a brushwood peat is replaced by a *Molinia-Sphagnum* peat, though here pollen dating is absent. In this peat occur the horizontal *Quercus* trunks referred to before. They may represent the last survivors of the marginal trees which were overwhelmed by the extension of the *Sphagnum* peat. The lateral extension of this peat had largely taken place before the end of subzone F 2. In this subzone *Sphagnum* peat appears at the side of the Teifi in diagram S.E. 18, and doubtless the lateral extension of the western bog to the river dates from this period also. In this subzone the *Sphagnum* peat extends over the *Molinia-Sphagnum* peat and further up the hillside on either margin of the valley. However a narrow belt with *Molinia* seems always to have insulated the raised bog proper from the hillside.

The *Sphagnum* peat referred to above was everywhere highly humified and of a dark brown colour, save where some content of *Molinia* lightened the colour. In some marginal layers it had also some content of wood, mainly *Betula*. Resting on this peat, and separated from it by an abrupt transition, was a fresh slightly humified light-coloured *Sphagnum* peat, which nowhere contained any appreciable quantity of wood. This transition was taken as the basis for separating zone F from zone G. A similar division of the *Sphagnum* peat into two well-marked types, a lower highly humified peat and upper less humified peat, has long been known in the raised bogs of north-west Europe, and has been recognized in the British Isles by Erdtman (1928). (The authors, however, cannot agree, especially in regard to these bogs, with his statement that the junction between these two peats in the British Isles is, as a rule, not clearly defined.) To this well-marked horizon the term "Grenz" has been given, and it is always regarded as due to an abrupt alteration in the conditions of peat formation. The initiation of the growth of this fresh peat is considered to have followed the climatic deterioration which has been recorded in north-west Europe, and there marks the beginning of the sub-Atlantic, the latest of the periods into which post-glacial time has been divided. These divisions were first established by Blytt and Sernander towards the end of the last century, and have

been supported by ever increasing evidence produced by later workers.

In this fresh *Sphagnum* peat above the "Grenz" a thin layer of more highly humified peat could be traced throughout the western and the south-eastern bogs, and was also observed in the north-eastern bog. It was, as noted above, particularly striking in the marginal cuttings, where it appeared as a darker band in the fresher peat above and below it. In those turves which included the band as a central layer, the more humified peat shrank considerably on drying, much more so than the neighbouring layers, and so a "waisted" appearance developed. For illustration of both these features see Plate VI. In the zonation previously suggested for the diagrams, this layer forms zone H, and the fresh peat above it zone J.

The widely extended occurrence of this feature in all three bogs makes it impossible to consider it as a local feature of an individual bog, and suggests that its nature may be similar to the bands of more highly humified peat, occurring in the upper fresh *Sphagnum* peat, which have been recorded by Granlund (1932) in the bogs of south-east Sweden. Granlund suggests that such layers are due to slowing up of bog growth by unfavourable conditions, and to the level marking the sudden renewal of growth he gives the name "Rekurrenzflache", which has been translated by Jessen as Recurrence-surface. In the field however, in this instance, the layer of highly humified peat was sharply set off from the fresher peat above and below, and was much more prominent than the "surface" itself, and it would here seem preferable to recognize the *layer* as the unit, which may be called the *Retardation layer*, to express its slower rate of formation.

Granlund has discussed the causes underlying these retardations and renewals of peat formation, and considers that variation in rainfall is the most important factor, increased growth following increased precipitation. The "Grenz" itself he regards as the most important of the five such Recurrence-surfaces, which he has found throughout the bogs of south-west Sweden.

(*b*) The successive zones which were recognized by correlation between the borings and the pollen diagrams have been shown to give a consistent basis for an interpretation of the development of the bog. At the same time it is likely that at least some of them have a wider significance. Thus the Grenz-horizon, used as the border between zones G and F, has already been suggested to correspond to

the sub-Boreal sub-Atlantic climatic change: the retardation layer is also probably an effect of climatic change recognizable widely beyond Tregaron itself. These are stratigraphic indices, but in the lower parts of the diagram the zones, established on a basis of pollen composition alone, can be referred to a wider importance. The transition from zone E to zone F is an horizon found throughout western Europe, and occurring in north-east Ireland also (see diagram by Jessen 1936). It is always marked by a diminution in the quantity of *Corylus*, and a sudden expansion of *Alnus* at the expense of *Betula* and *Pinus*. There can be little doubt that this horizon marks the Boreal-Atlantic transition. In these diagrams there is no basis for splitting zone F into two parts to correspond with the Atlantic and sub-Boreal periods.

The rough correspondence of zone E with the Boreal period is clear, since here *Ulmus* and *Quercus* first extend, and here occur the typically high *Corylus* values. The applicability of the subzones of E beyond Tregaron is uncertain. It seems very likely that the onset of the Boreal period is seen only at S.E. 10, where zone D has been separated at the base. Here *Quercus* and *Ulmus* are quite absent, and *Corylus* is still very sparse, *Betula* and *Pinus* contributing all the pollen. There is little doubt that zone D represents the end of the pre-Boreal period.

The authors suggest tentatively the following correlation with the numbered zones used by Jessen (1935) for Denmark, the nearest country for which established zonation of the pollen diagrams has been published.

Tregaron	Denmark (Jessen)	Approximate climatic periods (as in Jessen)
J	—	
H	ix	Sub-Atlantic
G	—	
F	viii	Sub-Boreal
	vii	Atlantic
E	v and vi	Boreal
D	iv	Pre-Boreal

Though Erdtman (1928) has not published a diagram from the bog, he has divided the deposits into three zones, post-Boreal, Boreal, and partly pre-Boreal. As far as his published results allow comparison, his pre-Boreal appears to correspond with zone D of this paper, his Boreal with zone E, while his post-Boreal embraces all the later zones.

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EXPLANATION OF PLATE VI

Phot. 1. View from the hill-side above Maes Llyn cottage, looking south-east over the valley. In the left distance the moraine on which is Tregaron village; in the middle foreground the S.E. bog with marginal peat cuttings, and in the right distance the Teifi with the foot of the W. bog beyond it. [Phot. H. Godwin.]

Phot. 2. A peat cutting profile on the S.E. bog. Just above the water line a paler line shows the band of greasy humified peat which is the retardation layer. Above and below it is fresh *Sphagnum* peat. The cut turves which rest on the surface have happened to be cut across the retardation layer, and in drying the peat of this layer has shrunk and cracked far more than the fresh *Sphagnum* peat. This has given each turf a "waisted" shape, which indicates how clearly the layer can be set off in appearance and behaviour. [Phot. F. J. North.]

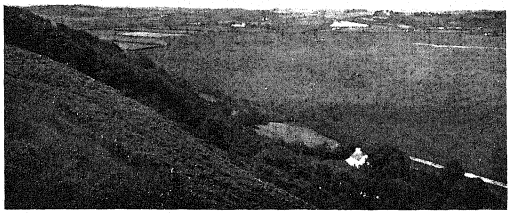

FIG. 1.

FIG. 2.

GODWIN AND MITCHELL—STRATIGRAPHY AND DEVELOPMENT OF TWO RAISED BOGS

THE RESPIRATORY DECOMPOSITION OF PYRUVIC ACID BY BARLEY

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(With 9 figures in the text)

THERE are now good grounds for supposing that acetaldehyde is formed in the higher plants and is either oxidized aerobically or reduced anaerobically. The evidence (reviewed in Thomas, 1935) has been obtained from a number of different plants by the efforts of independent workers, but the immediate origin of the acetaldehyde has not hitherto been made certain. On account of the analogy from yeast and the commonly accepted theories of alcoholic fermentation (both of Neuberg and of Meyerhof), pyruvic acid may be regarded as a very promising candidate for the position. It is not the only possibility, however, and acetaldehyde is definitely known to arise from other sources in the metabolism of bacteria (Stevenson, 1930). The decomposition of pyruvic acid and its connexion with the respiratory system in one of the higher plants, barley, is dealt with in this paper.

The pyruvic acid used was from a sample supplied by the B.D.H. As this was concentrated it was likely to contain the condensation product α-keto-γ-valerolactone-γ-carboxylic acid (Clift & Cook, 1932). To remove this the stock acid was redistilled *in vacuo* (B.P.$_{12}$ 65° C.). About two-thirds of the sample distilled over leaving a dark syrupy liquid fitting Clift & Cook's description of the valerolactone. Attempts at further purification by crystallizing out the acid failed. After desiccation over calcium chloride for 3 days *in vacuo* a sample of the redistilled acid was analysed. The acid value was 95·90% of the theoretical and the carbonyl (bisulphite fixation) value 99·65%. The purified acid was kept as an $M/10$ solution at which concentration it is stable.

[1] Rhodes Scholar, 1933–6.

EXPERIMENTS WITH POWDERED BARLEY. CARBOXYLASE

Method. The aim of the experiments in this section was to discover whether barley tissues contain an enzyme capable of breaking down pyruvic acid. The use of dead material avoids a number of difficulties and is a useful preliminary to the study of the living system; the latter is undertaken in the next section. The easiest decomposition product of pyruvic acid to detect and estimate is carbon dioxide, and its formation under suitable conditions may be taken as a satisfactory criterion of breakdown. In the following experiments examination of the carbon dioxide release was carried out in the fermentation apparatus already described by James & James (1936). The prepared barley powder was put into the fermentation tube together with 15 c.c. of pyruvic acid ($M/10$ or $M/15$); 1 c.c. of phosphate buffer to give a pH 5·7, and a few drops of thymol dissolved in alcohol. A control tube was set up at the same time having 15 c.c. of distilled water instead of the pyruvic acid but otherwise identical with the first. In the earliest experiments the tubes were filled with carbon dioxide-free nitrogen, but in later experiments this was replaced by carbon dioxide-free air and a few experiments were also performed *in vacuo*. When ready the two tubes were placed side by side in a thermostat bath at 30° C. and shaken for 3 hr., or occasionally longer. At the end of this time they were removed and the carbon dioxide estimated according to the usual method (James & James, 1936, p. 7).

Sterility. It was found in preliminary experiments that bacterial contamination was very obvious after 12–24 hr., so very short periods, usually 3 hr., were used. Bacterial counts during the first few hours of incubation at 30° C. were carried out by Miss J. M. Cragg by means of plating experiments. She found that material prepared in various ways from seedlings with expanded leaves was invariably contaminated at the start of the experiment. With a neutral buffer the number of bacteria usually increased by about 60 % during the first 2 hr. whether thymol was present or not. After 4 hr. no bacteria survived if thymol was present, and about half the original number in its absence. The addition of pyruvate decreased the number of viable bacteria after 2 hr. and made no difference after 4. An acid medium reduced the number of bacteria markedly; at pH 4·4 the experiment was virtually sterile after 2 hr., there being no period of increase as at pH 7. Our experiments carried out at pH 5·7 cannot therefore claim complete sterility, but it is evident that there was no

considerable increase of bacteria during the incubation such as might lead to serious interference. The bacterial counts were all made on material derived from seedlings with expanded leaves. Young sprouts, removed after a few hours from the endosperm, would be expected to show less infection on account of the smaller surface exposed and the shorter time of exposure, both during growth and drying. Hence, if any considerable proportion of the carbon dioxide collected was of bacterial origin, the oldest material would give the most. Actually, as the following results show, it gave the least.

Results

Liberation of carbon dioxide. The powder dried at room temperature was derived from young plants of various ages from 4 hr. to 10 days. When sprouting grains (4–90 hr. at 21° C. after addition of water) were to be examined only the embryonic tissues were utilized. The removal of the endosperm, with its low respiratory rate, increased the amount of activity that might be expected from a given bulk of material. The chances of loss of pyruvate by adsorption on inert surfaces was thus greatly reduced, as well as the possibility of carbon dioxide production from other respirable substances from the storage tissues. As a result of this treatment the control tubes only showed a very small production of carbon dioxide. The results of all the experiments carried out with sprouting grains are given in Table I.

TABLE I

			CO_2 produced in mg.		
Exp.	Age of embryos in hr.	No. of embryos per tube	Control	With pyruvic acid	Difference per 100 embryos
1	4	275	0·76	4·78	1·45
2	24	300	0·78	5·46	1·56
3	24	350	1·08	7·94	1·97
4	48	95	0·43	1·58	1·21
5	60	150	1·19	2·21	1·02
6	66	100	0·29	1·83	1·54
7	90	100	0·54	1·83	1·29
				Mean	1·43

The fermentation time was 3 hr. in each experiment.

In the two following experiments seedlings, 5 and 10 days old respectively, were used. When the 5-day-old seedlings were harvested the remains of the grain were removed and the rest of the tissues dried for 4 days at 30° C. The dried material was then powdered and

used exactly as in the previous experiments. The 10-day-old seedlings were raised in a greenhouse instead of an incubator, and were therefore green instead of etiolated. Satisfactory drying becomes a prolonged process with such material, and as this might involve serious contamination the seedlings were crushed and roughly dried in a hydraulic press at 2 tons/sq. in. The resulting powder was utilized as before. The carbon dioxide produced from 15 c.c. of $M/15$ pyruvic acid is given below.

TABLE II

Exp.	Age of seedlings in days	No. of seedlings per tube	CO_2 produced in mg. Control	CO_2 produced in mg. With pyruvic acid	Difference per 100 seedlings
8	5	100	0·16	1·20	1·04
9	10	60	0·25	0·84	0·99

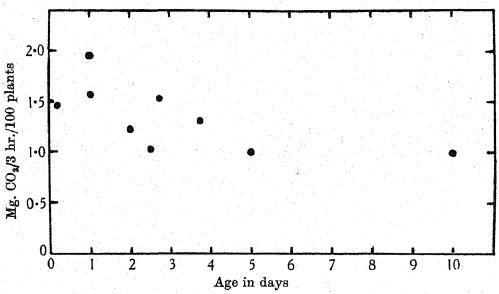

Fig. 1. Carbon dioxide released from pyruvate by powdered barley seedlings of various ages.

The results given in Tables I and II are summarized in Fig. 1. It is interesting to note that the activity, which from the following experiments may be ascribed to the enzyme carboxylase, is either already present in the dormant seed, or arises with astonishing rapidity during the act of soaking. In this it resembles the liquefying and saccharogenic enzymes of barley amylase. The apparent slow decline of the carboxylase activity from an initial maximum may be due to the development of an inhibitor rather than to actual breakdown of the carboxylase. In an experiment (no. 10) where fifty

6-day-old seedlings were added to a hundred 4 hr. embryos the carbon dioxide output was lowered to 0·71 mg. If the fifty seedlings are credited with 0·50 mg. (see Table II) this only leaves 0·21 mg. for the 100 embryos, a very much lower value than those of Table I. It does not necessarily follow that the inhibitor would be effective in living cells, and in any case it clearly does not have the capacity to suppress carboxylase activity entirely.

Since it has been shown that non-enzymes such as aniline and related compounds are active in decarboxylation (Wetzel, 1931), the following experiment was performed to test the heat-sensitive (enzymic) nature of the barley system.

Exp. 11. A sample of 380 embryos was dried for 12 hr. at 30° C. after 24 hr. in contact with moisture. They were then powdered and divided into two equal parts by weighing. Two tubes were prepared as follows:

(*a*) 15 c.c. *M*/15 pyruvic acid + 1 c.c. phosphate buffer + thymol + 0·26 g. barley powder. Carbon dioxide formation was estimated in the usual way.

(*b*) 10 c.c. distilled water + 0·26 g. barley powder. Boiled for 10 min. and cooled. 5 c.c. *M*/5 pyruvic acid + 1 c.c. phosphate buffer + thymol then added and carbon dioxide output estimated as usual.

Tube *a*, unboiled, yielded 1·50 mg. Co_2 in 2 hr.

Tube *b*, boiled, yielded 0·14 mg. CO_2 in 2 hr.

Difference 1·36 mg. CO_2 in 2 hr.

The additional carbon dioxide produced in the unboiled tube is equivalent to 1·74 mg. per 100 embryos per 3 hr. This is exactly equal to the average of Exps. 2 and 3 with 24 hr. embryos. In other words there are no heat-stable substances in barley capable of decarboxylating pyruvic acid, all the carbon dioxide produced being accounted for by the heat-sensitive system.

In addition to the foregoing material obtained by drying, it was found possible to prepare an active powder from 5-day-old seedlings by treatment with acetone in a manner similar to the preparation of zymin from yeast. The seedlings were chopped up and immediately crushed under the antiseptic. After the material had been well stirred the acetone was drawn off, and a second quantity added. This in turn was followed by two washings with ether and the powder left at 30° C. overnight to remove the last traces of the liquid. About 3 g. of dry powder resulted from roughly 800 seedlings. Under conditions similar to those of the following experiments 1 g.

of powder (≡about 260 seedlings) released 0·78 mg. carbon dioxide from the pyruvic acid in 3 hr. The acetone preparation is thus clearly capable of decarboxylating pyruvic acid, but has only about one-quarter the activity of the air-dried material. This agrees with Wetzel's observations on yeast, but the activity of the barley powders, however prepared, is greatly inferior to those from yeast.

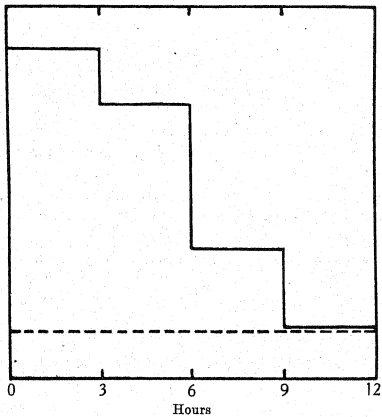

Fig. 2. Relative carbon dioxide release from pyruvate in successive three-hour periods (continuous line). Powder prepared from seedlings 24 hr. old. The dotted line represents an approximate control value without pyruvate in the mixture.

A sample of 1000 dried embryos was found to weigh 0·807 g. whence the maximal rate of decarboxylation (shown by Exp. 3) may be calculated as 8·2 mg. CO_2/g./hr. For comparison an experiment using 0·5 g. of pressed and dried yeast was carried out in the usual manner. It was found advisable to cut down the incubation time to 45 min., but in all other respects the experiment was identical with those already described. The decomposition of pyruvic acid amounted to 60·0 mg. CO_2/g./hr. The embryonic barley tissues thus show only about 12% of the activity of yeast weight for weight.

The enzyme in the barley powders rapidly loses activity in the presence of water. Fig. 2 gives a rough time curve of inactivation of

a powder prepared from 24 hr. embryos by the usual method of drying. It is possible that the initial activity is considerably higher than any actually recorded, but a reading obtained in 45 min. would have been very unlikely to approach the value given by yeast.

Formation of acetaldehyde. In a number of the foregoing experiments in which embryonic material was used, the Rimini test for acetaldehyde was applied to the digests; it always gave positive results. Since the evacuation of carbon dioxide for estimation necessarily removed any acetaldehyde present, the digests were returned to the thermostat bath for a further period of 3 hr. and the acetaldehyde test carried out without the removal of the carbon dioxide.

Experiments with living tissues

The experiments described in this section attempted to discover whether pyruvic acid can be introduced into living tissues and broken down by them as by the carboxylase preparations already described.

Germinating embryos, isolated from their endosperms, afford very suitable plant material for such experiments because they contain within themselves little respirable material, and when isolated and placed on moist sand or filter paper continue to give off carbon dioxide for 6 or 7 days at a slow and uniform rate. They will take up sugars and other substances added to the moist substratum, and utilize them in their metabolism.

In feeding such embryos with pyruvic acid it was decided to supply it dissolved in a normal culture solution rather than in pure water. It was hoped in this way to avoid, or at least minimize, any toxic effects that would have arisen in an unbalanced solution. For the same reason the strength of the pyruvic acid was limited to $M/20$.

Carbon dioxide emission and the respiratory quotient

The form of the chamber employed in these experiments is shown in Fig. 3. The Petri dish was first filled with fine silver sand purified by acid washing and afterwards sterilized by heating to 120° C. for 3 successive days. This sand was moistened with a solution made up by mixing equal parts of culture solution and $M/10$ pyruvic acid. The culture solutions had the final composition per litre (in g.):

$CaSO_4.2H_2O$	0·25	NaCl	0·08
$CaH_4(PO_4)_2H_2O$	0·25	KNO_3	0·70
$MgSO_4.7H_2O$	0·25	$FeCl_3.6H_2O$	0·005

It was made up at double strength for these experiments to allow for the dilution with pyruvic acid; in the control experiments run simultaneously it was diluted with an equal quantity of distilled water. The pH of this solution was given by the Hellige comparator as 5·0. No precipitation occurred when the solutions were autoclaved

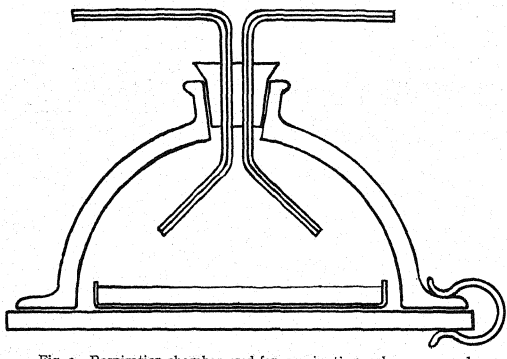

Fig. 3. Respiration chamber used for germinating embryos on sand moistened with prepared solutions.

immediately before use. After addition of pyruvic acid the reaction was readjusted to pH 5 by cautious addition of a few drops of normal NaOH. A preliminary experiment showed that, in the absence of any plant material, there was no appreciable breakdown of pyruvic acid to give carbon dioxide under the conditions employed.

The germinating embryos were obtained from grains that had been moistened with water for 4 hr. They were detached from their endosperms on the point of a flamed scalpel and immediately placed on prepared sand. 110 embryos were put in the pyruvate dish and an equal number in the control. The chambers, already sterilized by washing with alcohol and subsequently removing it with a sterilized air stream, were immediately closed over the Petri dishes and immersed in a thermostat bath at 21° C. The chambers were connected with an apparatus that drew continuous air streams through them, and allowed the carbon dioxide output to be measured in successive 6 hr. periods by the Pettenkofer method. The two

emission curves are shown in Fig. 4. At the end of the experiment both lots of embryos showed a 90% germination and an absence of any fungal infection.

In a second experiment two lots, each of 100 embryos, were similarly prepared and set up, but the plant chambers were connected to an apparatus that made an automatic collection of gas

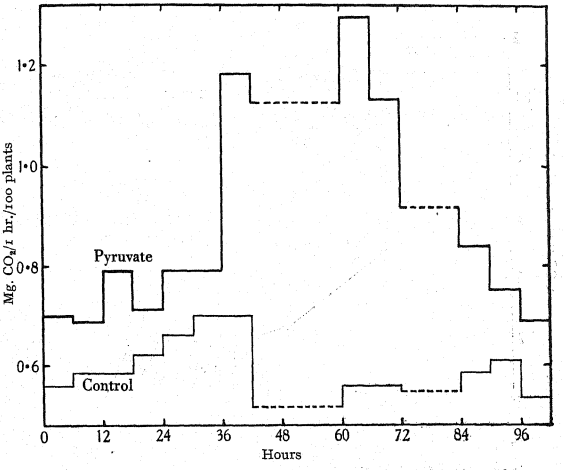

Fig. 4. Rate of carbon dioxide emission of embryos germinating on culture solution with *M*/20 pyruvate (thick line) and without pyruvate (thin line). Six-hour periods except as shown by broken line.

samples and permitted a continuous series of oxygen determinations to be made (at 2 hr. intervals) in addition to those for carbon dioxide. Gas analysis was carried out by the standard method of Haldane. The carbon dioxide emission curves (Fig. 5) differ hardly at all from those of the previous experiment (Fig. 4), and it seems evident that an increased carbon dioxide output results from the presence of the pyruvic acid. The intake of oxygen, on the other hand, shows a well-marked diminution leading to an increased value of the CO_2/O_2 ratio. To understand this it is necessary to mention that it

has been shown (A. L. James, unpublished) that barley embryos at this stage of development normally exhibit an extremely low respiratory quotient. Fig. 6 shows that the present experiment is no exception to the rule. The complete oxidation of pyruvic acid would give a CO_2/O_2 ratio of 1·2, and any decarboxylation of pyruvic acid without subsequent oxidation of the products would increase the

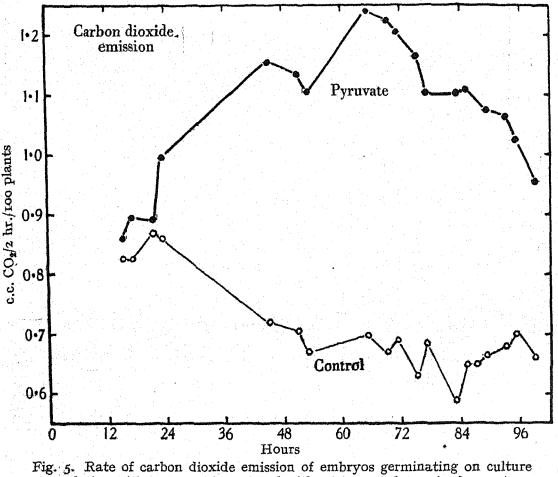

Fig. 5. Rate of carbon dioxide emission of embryos germinating on culture solution with pyruvate (•—•) and without (o— o) determined over two-hour periods.

value. In the present experiment the ratio in the presence of pyruvic acid flutuates from 0·93 to 0·66 in contrast with the values 0·47–0·23 in its absence. The fact that the pyruvate value does not reach the theoretical 1·2 may be ascribed to a persistence in part of the processes responsible for the low quotients in its absence. It is significant that the quotient produced by the fed seedlings tends to all simultaneously with that of the starved seedlings.

Similar feeding experiments were carried out using young leaves cut from seedlings raised in a greenhouse. Forty leaves were placed in a cylindrical chamber with their lower cut ends dipping into 5 c.c. of

the culture + pyruvate solution. A little liquid paraffin was run over the surface of the solution to prevent infection and a consequent gas exchange due to moulds or bacteria. The pyruvate in this experi-

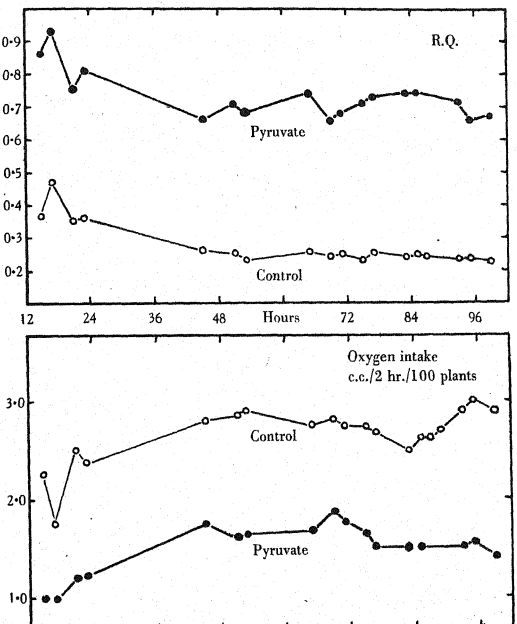

Fig. 6. R.Q. (upper panel) and oxygen intake (lower panel), corresponding with Fig. 5.

ment was derived from a commercial sample of sodium pyruvate instead of the purified pyruvic acid used in the other experiments. The carbon dioxide emissions of the pyruvate and a corresponding control tube are shown in Fig. 7. The experiment was stopped after 24 hr.; and examination then showed that the leaves remained

perfectly healthy in appearance, and those receiving pyruvate could not be distinguished by inspection from the controls; there was no sign of infection in the pyruvate solution.

In a further experiment two batches of fifty leaves were enclosed in the respiration chambers with their ends dipping into distilled water. Their carbon dioxide emission was determined over the next

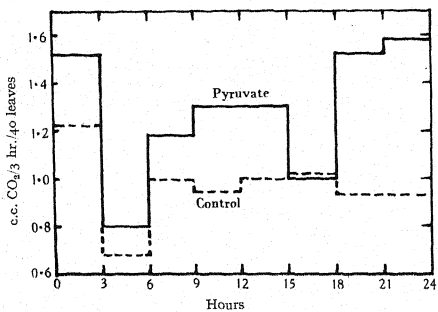

Fig. 7. Rate of carbon dioxide emission of young leaves absorbing solutions with pyruvate (continuous line) and without (broken line).

6 hr. and the leaves then withdrawn. The batch showing the faster emission was allowed to take up culture solution through the cut ends for 45 min. and the other batch was similarly allowed to absorb culture solution + pyruvic acid. During this period they were placed under a bright light to promote transpiration, and a side experiment with a dye solution showed that uptake was vigorous and that the solution thoroughly permeated the leaves. Both batches were then returned to the respiration chambers and the carbon dioxide emissions determined. The results are shown in Fig. 8 A and those of a similar experiment with more frequent readings in Fig. 8 B. There was no free pyruvate solution in the respiratory chambers in these two experiments.

It seems evident from these results that leaves, like young embryos, increase their carbon dioxide output when supplied externally with pyruvic acid. There is also a rise in the respiratory quotient though it is not so noticeable as in embryos, since the normal quotient is itself higher. In an experiment using green leaves from

17-day-old seedlings the average CO_2/O_2 ratio was 0·94 in the presence of pyruvic acid and 0·74 in its absence. The fact that the control value falls so far below unity indicates that the leaves had very little initial carbohydrate, and, as is usual under such conditions, the individual values of the R.Q. were somewhat erratic (see Fig. 9).

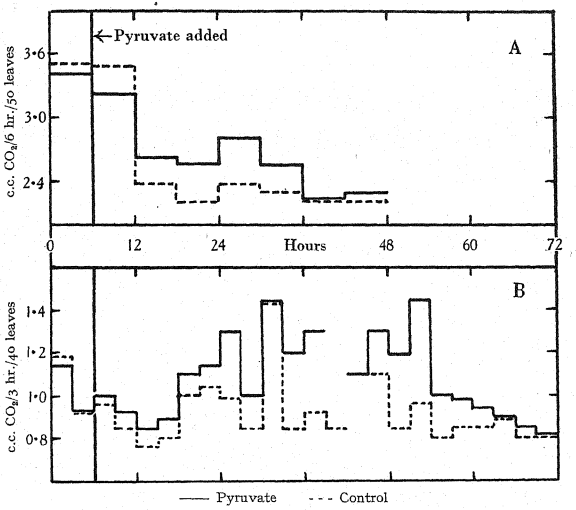

Fig. 8. Two experiments (A and B) showing the rate of carbon dioxide emission by young leaves allowed to take up *M*/20 pyruvate for 45 mins. (continuous line). Controls without pyruvate shown by broken line.

Origin of the additional carbon dioxide

The pyruvic acid did not cause any visible damage to the leaves, and there seems no reason to doubt that the additional carbon dioxide was produced by its decarboxylation in the living cells. The embryos, on the other hand, did show certain abnormalities in the presence of the acid. These were examined in more detail in an experiment using two sets of forty embryos growing in sand, which was moistened with

the usual solutions. Instead of the Petri dishes being enclosed in respiration chambers, however, they were kept in this experiment under inverted filter funnels in a dark room, so that the embryos could be inspected at frequent intervals. The germination was between 80 and 90% in both dishes, but the roots formed in the

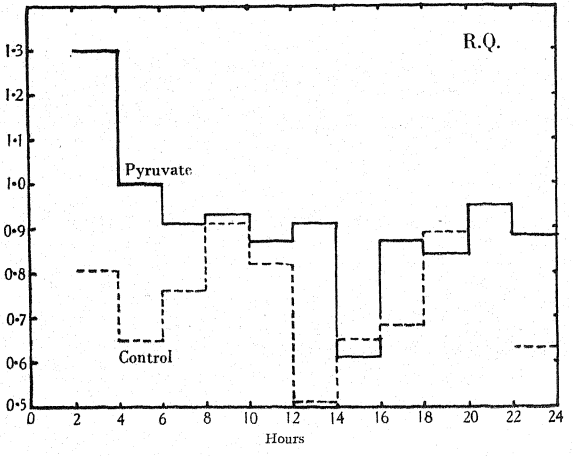

Fig. 9. R.Q. of young leaves absorbing solutions with pyruvate (continuous line) and without (broken line).

pyruvate culture did not penetrate freely into the sand like those of the controls. They remained on the surface and produced relatively few root hairs; the emerging coleoptiles did not elongate as rapidly as those of the controls, and tended to drag on the surface of the sand, instead of showing the normal geotropic response. Taken with the reduced absorption of oxygen shown by the second embryo experiment (Fig. 6), these symptoms seem to indicate that the normal oxidative syntheses characteristic of this phase of growth are seriously interfered with, though respiration as indicated by the carbon dioxide output goes on at an accelerated rate. There is some evidence that pyruvate also inhibits the anaerobic reduction of

methylene blue in barley (Miss Cragg, unpublished). It is likely therefore that the mechanism of this effect is an inactivation of a dehydrogenase by the excess pyruvate.

On account of these abnormalities shown by the fed embryos, it might be supposed that the additional carbon dioxide did not come from the breakdown of the pyruvic acid, but from the accelerated breakdown of substances already in the embryos. At least some of it must have come from the pyruvic acid, however, because the amount of carbon dioxide collected would have accounted for nearly the entire weight of the seedlings. Actually, they contained little reserve material, the endosperms having been removed, and consisted largely of non-respirable substances, such as cellulose, which were still present at the end of the experiment. In addition, carbon dioxide was still being given off, and the amount collected was clearly not the greatest possible. A rough calculation gives the following figures: the initial dry weight of 110 embryos is 73 mg., and the final weight not less than one-half of this, or 37 mg. The loss, using the conventional factor derived from the hexose formula, is equivalent to 50 mg. of carbon dioxide, whereas during the same period 87 mg. of carbon dioxide had been collected. This is greatly in excess of the estimated loss and not far short of the carbon dioxide equivalent of the total weight, i.e. 100 mg. It therefore seems evident that, even in the embryonic seedlings, at least some of the increased emission of carbon dioxide could only have been the result of metabolic breakdown of the pyruvic acid.

Absorption of pyruvic acid

It was shown by two separate methods that pyruvic acid actually penetrated into the tissues and was broken down there. The tests employed were (1) the iodoform and (2) the nitroprusside reactions.

(1) The iodoform reaction is given in general by compounds with the group $CH_3.CO.C$, but also by ethyl alcohol, the detection of which is the most familiar biochemical use of the reaction. Before it could be used for the demonstration of pyruvic acid it was, therefore, necessary to be certain that no alcohol was present in the tissues before or after the application of the acid. Extracts from untreated tissues invariably failed to give any iodoform, and the absence of alcohol after treatment with pyruvic acid was shown as follows. About a thousand seeds were germinated on filter paper moistened with $M/10$ pyruvate solution. They were kept at 21° C. for 4 days,

by which time they had sprouted. They were next well washed and crushed up, and the mash distilled at 65–70° C. under reduced pressure. The distillate was tested for alcohol, but no iodoform was obtained. In contrast with this volatile fraction water extracts of seedlings, that had been allowed to take up pyruvate, always gave a strong reaction. Portions that had come into direct contact with the solution were discarded so it must be assumed that the reaction was due to pyruvate that had entered the tissues. It should, perhaps, be mentioned that all these experiments were carried out in air; in the absence of oxygen alcohol formation would of course have been more likely to interfere.

(2) The nitro-prusside reaction, which is highly sensitive and specific for pyruvic acid, was tested for our material as follows. One drop of $M/12$ pyruvic acid was pipetted into a test-tube containing about 5 c.c. of distilled water. A few drops of concentrated sodium nitro-prusside were added, followed by a liberal quantity of strong ammonia. The solution darkened on standing, and the red-brown of the nitro-prusside slowly gave place to green; a red colour was produced by adding caustic soda, which returned to green when treated with acetic acid. Grinding up leaf material with the pyruvic acid did not interfere with any of these reactions, so that, if pyruvic acid were present naturally, it should be revealed. Actually no reaction is given by leaves taken straight from the plant, but the penetration of the pyruvate into treated leaves was shown to occur in the following experiment.

Twenty young leaves were cut from seedlings and immediately placed with their cut ends dipping into a neutralized $M/12$ pyruvate solution. They were left standing in a greenhouse for $1\frac{1}{2}$ hr., after which they were removed and those parts that had been submerged in the solution cut off. The remainder of the leaves was chopped up, and ground to a fine mash in trichloracetic acid; the extract was filtered off, a clear filtrate being obtained. This was tested and a clear reaction for pyruvic acid resulted exactly as described above.

The destruction of pyruvic acid

Having shown that pyruvate was absorbed it was next necessary to discover whether it was broken down in the respiring tissues. To do this an experiment, similar to the last, was carried out using two batches each of twenty-five leaves. The first batch was tested immediately after the absorption period of $1\frac{1}{2}$ hr., and the presence

of pyruvate confirmed. At the same time the second set of leaves was taken from the pyruvate solution and transferred, after the lower ends had been well washed, to distilled water. It was left in a dark incubator at 21° C. for 6 days and then tested for pyruvate. Both the nitroprusside and the iodoform reactions gave entirely negative results, showing that complete breakdown had occurred by this time.

Discussion

So far as we are aware, pyruvic acid has never been isolated from barley or any other of the higher plants, although Neuberg's fixation of calcium pyruvate from a fermenting yeast preparation is well known. No direct evidence of its presence in our plants could be obtained; the nitroprusside reaction which might have been expected to reveal a concentration of $M/1000$ or less, consistently gave negative results. These facts may arise from a rapid decomposition just as readily as from a negligible rate of formation, and in view of the other data presented rapid breakdown seems the more likely alternative. There also remains the possibility that more searching methods would reveal its presence in small amounts.

The existence of carboxylase in some of the higher plants has already been announced, but it seems rather doubtful whether the precautions against bacterial interference have always been adequate. Bodnar (1916), although he used experimental periods up to 60 hr., was satisfied that the pressed juice of potatoes and sugar beet remained sterile and was capable of carboxylase activity. We were unable in our own experiments to maintain sterility for so long a time. Bodnar & Hoffner (1925) were able to confirm the earlier results of Zaleski (1913) showing that pyruvate is decarboxylated by meal from peas, wheat and lupins.

The decomposition of pyruvic acid by barley in our own experiments is probably due to a carboxylase since carbon dioxide is given off in the absence of oxygen and acetaldehyde accumulates in the digests. In the living tissues of our plants we were never able to show the existence of acetaldehyde, even after pyruvate had been added and broken down. The Rimini test which we used gave positive results when very small quantities of acetaldehyde were added artificially but consistently negative results with the untreated tissues. In spite of this the intermediary formation of acetaldehyde in our plants may be inferred from the detection of acetoin, a simple condensation product (see James & James, 1936, p. 2); and

Niethammer (1928) claims to have detected acetaldehyde itself in germinating barley as well as other seedlings.

Acetaldehyde was formed in the digests in the presence of air so the carboxylase is capable of working under aerobic conditions. All our other results are also consistent with the view that the decarboxylation of pyruvic acid (probably followed by oxidation of the acetaldehyde) is a normal stage in the aerobic respiration of barley. It is, however, possible that when oxygen is admitted acetaldehyde is only formed in a side reaction, for there is much uncertainty about its immediate fate. Until we know definitely what substances are oxidized during respiration the exact importance of decarboxylation must be doubtful. That it can occur in the presence of oxygen and probably does occur at least to some extent seems to follow from our results.

SUMMARY

1. It is shown that young barley tissues, killed by drying, mechanical crushing or treatment with acetone, will break down pyruvic acid with the formation of carbon dioxide and acetaldehyde.

2. The activity is due to a carboxylase, which is destroyed by heat, rapidly loses activity when suspended in water, and appears liable to the action of a partial inhibitor present in some preparations.

3. The carboxylase remains active in the presence of oxygen.

4. Germinating embryos and young detached leaves, when supplied with $M/20$ pyruvic acid in culture solution, increase their rate of carbon dioxide emission.

5. In these feeding experiments the respiratory quotient rises, but does not reach 1·2. This is to be expected on the assumption that the pyruvic acid is being respired simultaneously with internal substances.

6. The pyruvic acid absorbed is shown to be broken down.

It is suggested from the above evidence that pyruvic acid is likely to be a normal intermediary in aerobic respiration.

It is a pleasure to acknowledge our indebtedness and express our thanks to Dr F. B. Hora for technical assistance, and to the Rhodes Trustees and the Department of Scientific and Industrial Research who helped us financially.

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PERENNATION IN *CUSCUTA REFLEXA* ROXB.

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(With 1 figure in the text)

Introduction

Cuscuta is a common parasite in several parts of India. Of the Indian species, *C. reflexa* Roxb. has a wide range of distribution extending sometimes up to 6000 ft. or even more above sea-level in the hills of South India (Gamble, 1921) as well as North India (Singh, 1933). All the Indian species of *Cuscuta* are described as annuals (Cooke, 1908) like most of the European species. Even though they are attached to perennial woody hosts, they die after flowering. Kerner & Oliver (1902) state that perennial species are found in the tropics, and mention as an example *C. Verrucosa*, the suckers of which "continue to exercise their function throughout the year, wherever they have once attacked the host". According to the *Index Kewensis* (1, 674) *C. Verrucosa* Sweet is a synonym of *C. reflexa* Roxb. *C. reflexa* growing on *Salvia* in a greenhouse at the Cambridge University Botanic Gardens has been mentioned by Thoday (1911) as a perennial species. In the United States of America *Cuscuta epithymum* on alfalfa has been described as a perennial which produces seeds only rarely, and the continuation of infestations is more often due to resumption of growth by overwintered stems than to the germination of new seed (Steward & French, 1909). It seems clear that in all these cases the parasite as a whole persists. No species of *Cuscuta* has so far been recorded as perennial in habit in India, and the mode of perennation to be described seems not to have been observed before.

Material and field observation

Material of *Cuscuta reflexa* Roxb., growing parasitically on species of *Strobilanthes* (*S. Gossypinus* and *S. Canaricus*) (Hooker, 1885), was collected in several infected areas of the Mysore Forests some 5000 ft. above sea-level. Observations recorded show that *Cuscuta* flourishes, attacking practically every host plant in the area by the end of the

south-west monsoon, i.e. October and November. The growth gradually declines during the next three months, when the parasite produces flowers and fruits in abundance. By the time the summer sets in, nothing remains of the parasite except occasional dried black pieces of its stem coiled round the host (Fig. 1).

This feature was observed year by year for a long time. Meanwhile, a search was made in the early part of the rainy season for germinating seeds and seedlings of *Cuscuta* in the areas where it had been found growing extensively in the previous season, but without success.

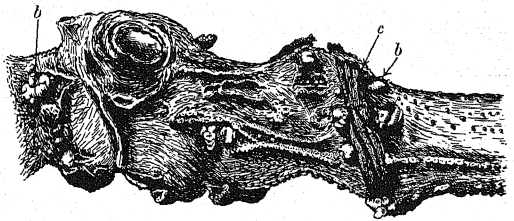

Fig. 1. A portion of the old stem of *Strobilanthes* with a black dead piece of *Cuscuta* stem (*c*), on either side of which young buds (*b*) have emerged to develop into new shoots. Higher up the stem are other *Cuscuta* buds arising from buried haustoria. × 2.

However, on one host plant a peculiar growth was noticed from which numbers of *Cuscuta* branches were spreading in all directions. Further observation revealed, in a number of instances, groups of young shoots of *Cuscuta* of varying lengths (1–20 mm.) emerging from the host stem, each one of them starting the attack independently. Subsequently, similar growths were found in all the areas on several hosts. Sometimes pieces of black dead stem of the previous growing season were found still adhering to the host stem with new young shoots of the parasite emerging on either side of them (Fig. 1).

Anatomy of the parasite during perennation

The mode of attack and the formation of the sucker in this species are similar to those described by Peirce (1893). After flowering, the parasite dies and the host repairs the wounds inflicted by the parasite. However, the haustorial tissues of the parasite often

persist in the body of the host during the dry months of the year. While the healing of the wound is going on, the subsequent secondary growth of the host not only encloses the absorbing tissue completely, but also breaks it up into a number of isolated groups or islands by penetrating into it. The islands are well protected and nourished by the host. They begin their activity only in the next rainy season, when they grow out of the host tissue, each forming a new shoot. From one sucker wound as many as 8–10 shoots may arise to renew the attack.

DISCUSSION

This type of perennation is different from the perennial habit in a uniform tropical climate or in a greenhouse, where the conditions favour indefinite continuance of growth. The perennation of *Cuscuta reflexa* Roxb. in the manner described appears to be an adaptation to the ecological conditions under which the parasite is obliged to grow. It could not continue its vegetative growth throughout the year by reason of the marked variations in climatic conditions, especially rainfall. Propagation by seed is rarely possible in view of the absence of suitable hosts at proper stages of growth in the neighbourhood of the seedling. It is necessary to remember in this connexion that so dense is the thicket of *Strobilanthes* in the undergrowth of forest that under ordinary natural circumstances, it is only at the time of the periodical flowering that the tree seedlings can establish themselves (Gamble, 1888). What is true of tree seedlings appears to be true for *Cuscuta* seedlings.

SUMMARY

A new method of perennation of *Cuscuta* has been observed where perennation takes place by the survival of only the haustorial tissue within the host during the dry months, the parasite body completely disappearing. The haustorial tissue gives rise to a number of young shoots early in the next growing season.

I wish to thank Prof. Dr R. Ruggles Gates, not only for his advice and assistance but also for encouragement in the publication of this paper, and Prof. D. Thoday who kindly read and criticized the manuscript before publication. I wish to express my thanks to Dr M. A. Sampathkumaran for his continued interest during this work.

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